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Vasodilator effects of adrenomedullin on small pulmonary arteries and veins in anaesthetized cats

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1 This study was conducted to determine adrenomedullin (AM) action sites in the pulmonary vascular bed and the relation between its vasodilator effects and vascular tone. Moreover, an examination was made into whether calcitonin gene-related peptide (CGRP) receptors mediate pulmonary vasodilatations induced by AM. To this end, we directly measured internal diameter (i.d.) changes in small pulmonary arteries and veins (100–1100 μm i.d.) by use of an X-ray televison system on the *in vivo* cat lung.

2 Under control (resting vascular tone) conditions, AM injections into the left main pulmonary artery caused dose-related i.d. increases in both small arteries and veins. The mean i.d. increase of the 100–1100 μm arteries (4 ± 1, 11 ± 2, and 17 ± 2% with 0.01, 0.1, and 1 nmol kg^{-1} AM, respectively) was significantly larger than that for the veins (1 ± 1, 5 ± 2, and 7 ± 2% with 0.01, 0.1 and 1 nmol kg^{-1} AM, respectively) whatever the injected dose of AM.

3 When unilobar hypoxia (5% O_2) had decreased the i.d. of the 100–1100 μm arteries and veins by 16 ± 3 and 6 ± 3%, respectively, AM (0.1 nmol kg^{-1}) was able to induce significantly larger i.d. increases in the arteries (28 ± 3%) and veins (11 ± 3%) than those under control conditions.

4 The AM-induced i.d. response pattern in the serially connected pulmonary arteries was quite different from that induced by CGRP; AM caused a greater increase in smaller vessels (100–500 μm) than in larger vessels (500–1100 μm). In the case of CGRP, a greater increase was observed in the larger vessels.

5 $CGRP_{8-37}$ (100 nmol kg^{-1}, i.v., followed by a continuous infusion of 0.2 nmol kg^{-1} min^{-1}) had no significant effect on the i.d. increase induced by AM (0.1 nmol kg^{-1}) in any serial segments of the arteries and veins.

6 The results indicate that, in the cat, AM induces greater vasodilatation in small pulmonary arteries and lesser vasodilatation in small veins, the maximum dilatation being in the more peripheral arterial segment (100–500 μm). The vasodilator effect of AM was enhanced when vascular tone was elevated. The data suggest that the AM-induced pulmonary vasodilatation is not mediated by CGRP receptors but by its own specific receptor.

Keywords: Adrenomedullin; calcitonin gene-related peptide; $CGRP_{8-37}$; hexamethonium bromide; pulmonary vasodilatation; hypoxic pulmonary vasoconstriction; small pulmonary vessels; vascular internal diameter

Introduction

Adrenomedullin (AM) is a newly discovered peptide isolated from human phaeochromocytoma cells (Kitamura *et al*., 1993a). It has been shown that large amounts of AM and AM mRNA are present in the lung (Kitamura *et al*., 1993a,b). This organ is also the most prominent site of AM binding (Owji *et al*., 1995) and AM receptor gene expression (Kapas *et al*., 1995). Moreover, circulating AM (Kitamura *et al*., 1994) is removed when it passes through the pulmonary vascular bed (Nishikimi *et al*., 1994; Yoshibayashi *et al*., 1994). These properties of AM raise the speculation that AM may play a role in the regulation of pulmonary circulation.

It has been shown from pressure-flow relationships that AM injections decrease pulmonary vascular resistance in a dose-dependent manner when acting under conditions of elevated pulmonary vascular tone (DeWitt *et al*., 1994; Lippton *et al*., 1994), but not under resting tone conditions (Lippton *et al*., 1994). However, questions remain regarding exactly which pulmonary vascular segment dilates in response to injected AM and whether pulmonary arterial and venous responses to AM depend on their degrees of vascular tone.

AM shares its structural homology with calcitonin gene-related peptide (CGRP) (Kitamura *et al*., 1993a,b). In the systemic vascular beds, it has been suggested that AM acts on CGRP receptors to exert its vasodilator effects. $CGRP_{8-37}$, a CGRP receptor antagonist, inhibited vasodilator responses to AM in the rat isolated perfused mesenteric vascular bed (Nuki *et al*., 1993) and coronary vascular bed (Entzeroth *et al*., 1994) and *in vivo* in the rat skin microvasculature (Hall *et al*., 1995). Thus, both AM and CGRP seem to activate CGRP receptors. However, it has recently been demonstrated that AM increases intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP) in rat cultured aortic smooth muscle cells via its specific receptors to evoke vasodilatation (Ishizaka *et al*., 1994). On the other hand, information on this problem in relation to the pulmonary circulation is very limited. While one research group has shown a significant effect of $CGRP_{8-37}$ on reductions in pulmonary vascular resistance due to AM injection in rat isolated lung (Zhao *et al*., 1996), another group has obtained no significant effect (Heaton *et al*., 1995). No large difference in vasodilator potency and duration of action has been shown between AM and CGRP in the cat perfused lung (DeWitt *et al*., 1994). However, until now, the questions as to how the pulmonary arterial and venous responses to AM are influenced by $CGRP_{8-37}$, and whether the vascular response pattern in the serially connected pulmonary arteries and veins is significantly different between AM and CGRP, have not been answered conclusively.

To resolve the above-mentioned issues, it is necessary to measure directly internal diameter (i.d.) changes in the small

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muscular pulmonary arteries and veins, which are highly responsive to various neurohumoral stimuli (Shirai *et al*., 1993; 1994a,b; 1996). In this study, using an X-ray television system on the *in vivo* cat lung (Sada *et al*., 1985), we quantitatively measured i.d. changes of small pulmonary arteries and veins (100–1100 μm i.d.) in response to AM injections. This was done for the small vessels with resting vascular tone and elevated vascular tone during alveolar hypoxia. Furthermore, to investigate whether the AM-induced i.d. response is mediated by CGRP receptor activation, the AM-induced i.d. response pattern in the serially connected pulmonary vessels was compared with that for CGRP, and then the effect of $CGRP_{8-37}$ on the AM-induced i.d. response pattern was examined.

Methods

Experimental procedure and angiography

The experiments were carried out on 15 cats (2.6–3.2 kg body weight) anaesthetized with sodium pentobarbitone (35 mg kg^{-1}, i.p.). The level of anaesthesia was maintained with supplemental doses of sodium pentobarbitone (2–3 mg kg^{-1}, i.v.) given at 0.5 to 1 h intervals. Each cat was intubated with an endotracheal tube and artificially ventilated with room air.

A catheter was introduced fluoroscopically from the right jugular vein into the left main pulmonary artery. A catheter was also inserted directly into the left atrium. Two additional catheters were then inserted into the right femoral artery and vein. Then, in order to expose the left lower lobe directly to the X-ray, the left-side rib cage (ribs 6–8) was partially excised. The end-expiratory pressure was adjusted to 4.0 cmH_2O to prevent lung collapse. Heparin sodium (500 iu kg^{-1}) was administered to prevent blood coagulation.

The system and experimental setup used for the angiography have been described in detail previously (Sada *et al*., 1985). Briefly, the cat was placed inside an X-ray apparatus box (Hitex) and fixed in such a manner that the exposed left lower lobe automatically came into contact with a plate just above the beryllium faceplate of an X-ray-sensitive, 1-in. vidicon camera (Hamamatsu Photonics). During temporary cessation of ventilation for 4–5 s at end expiration, contrast medium (3 ml, 60% Urografin) was injected into the main pulmonary artery at a constant speed (1.7 ml s^{-1}) and its passage through the pulmonary vascular bed was recorded serially at high speed (30 frames s^{-1}) on a video disk recorder (model VM-1000M, Victor) and a video tape recorder (model CR-850, Victor). During the experiment, the temperature in the box was maintained at 25–28°C and the surface of the exposed lung kept wet with warm (37°C) saline.

Analysis of i.d. response

Following the method described in our previous study (Shirai *et al*., 1994a), a random selection of many vascular sites for i.d. measurement was made. The percentage i.d. change in response to agents was calculated at each measured vascular site. The measured sites were classified into five vascular groups, i.e., 100–300, 300–500, 500–700, 700–900, 900–1100 μm, according to their baseline i.d. sizes, and the mean value of the i.d. percentage change was obtained in each group. By pooling of all the data, the mean value of the i.d. percentage change and i.d. absolute change for all the vessels ranging from 100 to 1100 μm i.d. was also obtained.

Experimental design

Protocol 1 The pulmonary vasodilator effects of AM under resting and elevated vascular tone conditions were studied in 6 cats. The baseline angiogram was recorded first and then AM (human, Peptide Institute) was injected into the left main pulmonary artery in doses of 0.01, 0.1 and 1 nmol kg^{-1}, in this order. The angiograms following the AM injections were taken 25–30 s after the end of the peptide injection, when pulmonary arterial pressure had decreased to nearly the minimum level. Thereafter, vasoconstrictor responses were induced by regional alveolar hypoxia. Details regarding the method of causing regional hypoxia have been described previously (Shirai *et al*., 1994a). Briefly, a concentric dual-lumen tracheal tube was inserted via tracheostomy. With the animal maintained on positive-pressure ventilation via the outer tube, the centre tube was extended into the main bronchus of the left lower lobe. It was possible to ventilate the lobe independently from the remaining lung by having the balloon cuff inflated around the tip of this centre tube. During the baseline condition, both the left lower lobe and the rest of the lung were ventilated with a gas mixture of ~40% O_2 in N_2. Following the baseline angiogram, hypoxia (5% O_2) was selectively administered to the left lower lobe for a period of 7 min. At the end of this period, the second angiogram with hypoxia was recorded. Then the third angiogram with hypoxia plus an AM injection was obtained. Hypoxia was exposed in the same manner as the case of the second angiography. AM (0.1 nmol kg^{-1}) was injected into the left main pulmonary artery 25–30 s before the end of the 7 min periods of hypoxic exposure and the angiogram was taken at the end of this period.

Protocol 2 The issue of whether CGRP receptors mediate the AM-induced pulmonary vasodilatation was examined in 5 cats. Assuming this to be the case, it would be expected that the AM-induced vasodilator pattern in the serially connected vessels would be similar to that for CGRP. Therefore, we examined pulmonary vasodilator responses to CGRP and compared them with AM-induced vasodilator responses obtained in protocol 1. Following the baseline angiogram, the angiograms with CGRP were recorded. Injection of CGRP (human, Peptide Insitute) into the left main pulmonary artery was performed in doses of 0.01, 0.1 and 1 nmol kg^{-1}, in this order. The angiograms with CGRP were taken in almost the same manner as those for AM. After the angiograms with CGRP were obtained, the effects of the CGRP antagonist, $CGRP_{8-37}$, on the AM-induced i.d. increase were examined. The baseline angiogram was taken ~5 min after the start of the administration of $CGRP_{8-37}$ (human, Peptide Institute; 100 nmol kg^{-1}, i.v., bolus, followed by an infusion of 0.2 nmol kg^{-1} min^{-1} throughout the experiment). The angiogram with AM (0.1 nmol kg^{-1}) was then recorded ~15 min after the baseline angiogram had been obtained. In preliminary experiments, the dose of $CGRP_{8-37}$ completely blocked i.d. increases due to intrapulmonary injections of CGRP (1 nmol kg^{-1}).

Protocol 3 In the present study, AM and CGRP injections significantly reduced systemic arterial pressure. It is therefore possible that arterial baroceptor reflex changes due to the blood pressure reductions have an effect on the i.d. increases caused by AM and CGRP. To examine this possibility, we measured i.d. responses to these peptides under conditions of ganglion blockade in 4 cats. The first paired angiograms before and after the injection of AM (1 nmol kg^{-1}) were taken ~10 min after the administration of a ganglion-blocking agent, hexamethonium bromide (5 mg kg^{-1}, i.v.). There was an interval of ~15 min between angiographies. Thirty to forty min after the first period angiograms, additional hexamethonium bromide (2 mg kg^{-1}) was administered to maintain ganglion blockade. Then the second paired angiograms before and after the injection of CGRP (1 nmol kg^{-1}) were recorded in a manner analogous to that for AM.

In preliminary experiments on 3 cats, we examined the time course of the i.d. increases in small vessels following AM and CGRP injections of 1 nmol kg^{-1}. With both the AM and CGRP, the i.d. increase almost reached a peak ~25–35 s after the injection had ended and returned to its initial baseline level ~15–20 min after the injection. The i.d. increase recorded

$\sim 10-15$ s after peptide injection was some 40–60% smaller than that recorded $\sim 25-35$ s after the injection, but in the case of the serial vascular segments the patterns were very similar. Therefore, in the present study the i.d. response following peptide injection was recorded only once, 25–30 s after the injection, at approximately the time of the maximum vasodilatation. Moreover, an interval of $\sim 30-40$ min between each peptide injection was allowed in order to eliminate any influence of the preceding peptide injection on the i.d.

Blood gases and pH were measured throughout the experiment by a blood gas analyser (ABL-2, Radiometer). During ventilations with room air, the P_{O_2}, P_{CO_2} and pH of the systemic arterial blood were 101 ± 3 mmHg, 31 ± 2 mmHg and 7.38 ± 0.02, respectively. In the hypoxic studies, before hypoxia the P_{O_2}, P_{CO_2} and pH were 255 ± 5 mmHg, 32 ± 2 mmHg and 7.37 ± 0.02. The hypoxic exposure decreased the P_{O_2} to 140 ± 6 mmHg but there was no change in P_{CO_2} and pH. Addition of AM further decreased the P_{O_2} to 98 ± 6 mmHg, but did not cause systemic hypoxaemia.

Statistical methods

The significance of differences in haemodynamic data between the conditions of baseline and of AM or CGRP injections was tested by analysis of variance (ANOVA) and Scheffe's test (Wallenstein *et al*., 1980) or by a paired *t* test. Differences in i.d. response between different doses of AM and CGRP, as well as between the five different-sized vascular groups, were assessed in the same way. Comparisons between the i.d. responses to AM in the absence and presence of $CGRP_{8-37}$ or hypoxia were made with an unpaired *t* test. Differences in i.d. values before and after AM or CGRP injection were examined by a paired *t* test. All results are expressed as mean ± s.e.mean. $P < 0.05$ was considered significant.

Results

Haemodynamic data

Mean pulmonary arterial pressure (PAP), mean left atrial pressure (LAP), and mean systemic arterial pressure (SAP) before and after AM or CGRP injection under the four different conditions are summarized in Table 1. These parameters were measured just before injection of the contrast medium. Under control conditions, AM and CGRP injections at a dose of 1 nmol kg^{-1} significantly decreased PAP by 0.7 and 0.6 mmHg, respectively. AM and CGRP injections in doses of 0.01 and 0.1 nmol kg^{-1} tended to decrease PAP, but this was not statistically significant. SAP was significantly decreased in response to AM and CGRP injections of 0.1 and 1 nmol kg^{-1}, but not in response to 0.01 nmol kg^{-1}. LAP remained unchanged whatever the dose of AM or CGRP.

Responses of i.d. to AM under control conditions and during hypoxic vasoconstriction

Figure 1 shows typical changes in i.d. in the small pulmonary arteries and veins in response to an AM injection. On injection, the i.d. of the arteries clearly increased in many branches, particularly in smaller branches. Increases in i.d. were also observed in many venous branches, but generally seemed larger in the arteries.

The mean value of the percentage change of i.d. for all 100–1100 μm vessels is shown in response to each of three different AM doses under control conditions (Figure 2). The mean baseline i.d. sizes of the arteries and veins studied for these data were 485 ± 18 and 497 ± 19 μm, respectively. Injections of AM 0.01–1 nmol kg^{-1} caused dose-related i.d. increases in the arteries. Venous i.d. also increased significantly with 0.1 and 1 nmol kg^{-1} AM, although not with the 0.01 nmol kg^{-1} dose. The i.d. increase was larger in the arteries than in the veins whatever dose of AM was injected.

The mean value of the i.d. changes (Figure 3a) and the mean value of the i.d. percentage changes (Figure 3b) for 100–1100 μm vessels in response to 0.1 nmol kg^{-1} AM are compared between the conditions of control and hypoxic i.d. reduction. The mean baseline i.d. sizes of the arteries and veins investigated were the same as those in Figure 2. The hypoxic i.d. reduction was 16 ± 3 and 6 ± 3% in the arteries and veins, respectively. Both the i.d. change and i.d. percentage change were significantly larger in the i.d.-constricted conditions than in the control conditions. This indicated that the AM-induced dilator effect on small pulmonary arteries and veins was enhanced in the presence of the hypoxic vasoconstriction.

Comparison of i.d. responses to AM and CGRP

CGRP injections, 0.01, 0.1 and 1 nmol kg^{-1}, increased the i.d. of 100–1100 μm arteries by 3 ± 1, 9 ± 2 and 15 ± 2%, respectively. Venous i.d. was also increased significantly by 3 ± 2 and 6 ± 2% with 0.1 and 1 nmol kg^{-1} CGRP; but not by the

Table 1 Haemodynamic data before and after adrenomedullin (AM) and CGRP injections into left main pulmonary artery

Condition	n	*PAP* (mmHg)	*LAP* (mmHg)	*SAP* (mmHg)
Control				
Baseline	6	16.1 ± 0.4	5.1 ± 0.3	102 ± 5
AM (0.01 nmol kg^{-1})	6	15.9 ± 0.4	5.1 ± 0.3	98 ± 5
AM (0.1 nmol kg^{-1})	6	15.7 ± 0.5	5.1 ± 0.3	89 ± 6*
AM (1 nmol kg^{-1})	6	15.4 ± 0.5*	5.0 ± 0.3	84 ± 6**
Baseline	5	16.3 ± 0.4	5.2 ± 0.3	100 ± 5
CGRP (0.01 nmol kg^{-1})	5	16.2 ± 0.4	5.2 ± 0.3	95 ± 5
CGRP (0.1 nmol kg^{-1})	5	16.0 ± 0.5	5.1 ± 0.3	90 ± 5*
CGRP (1 nmol kg^{-1})	5	15.7 ± 0.5*	5.1 ± 0.3	85 ± 6**
Preconstricted with hypoxia				
Baseline	6	16.8 ± 0.5	5.3 ± 0.3	104 ± 6
AM (0.1 nmol kg^{-1})	6	16.4 ± 0.5	5.3 ± 0.3	92 ± 6*
Pretreated with $CGRP_{8-37}$				
Baseline	5	16.4 ± 0.5	5.4 ± 0.3	105 ± 5
AM (0.1 nmol kg^{-1})	5	16.1 ± 0.5	5.4 ± 0.3	96 ± 6*
Pretreated with C_6				
Baseline	4	14.5 ± 0.5	5.0 ± 0.4	79 ± 6
AM (1 nmol kg^{-1})	4	13.5 ± 0.5*	4.8 ± 0.4	57 ± 6**
CGRP (1 nmol kg^{-1})	4	13.6 ± 0.5*	4.8 ± 0.4	59 ± 6**

Values are mean ± s.e.mean of *n* animals. PAP, mean pulmonary arterial pressure; LAP, mean left atrial pressure; SAP, mean systemic arterial pressure. C_6, hexamethonium bromide. Significantly different from baseline: *$P < 0.05$, **$P < 0.01$.

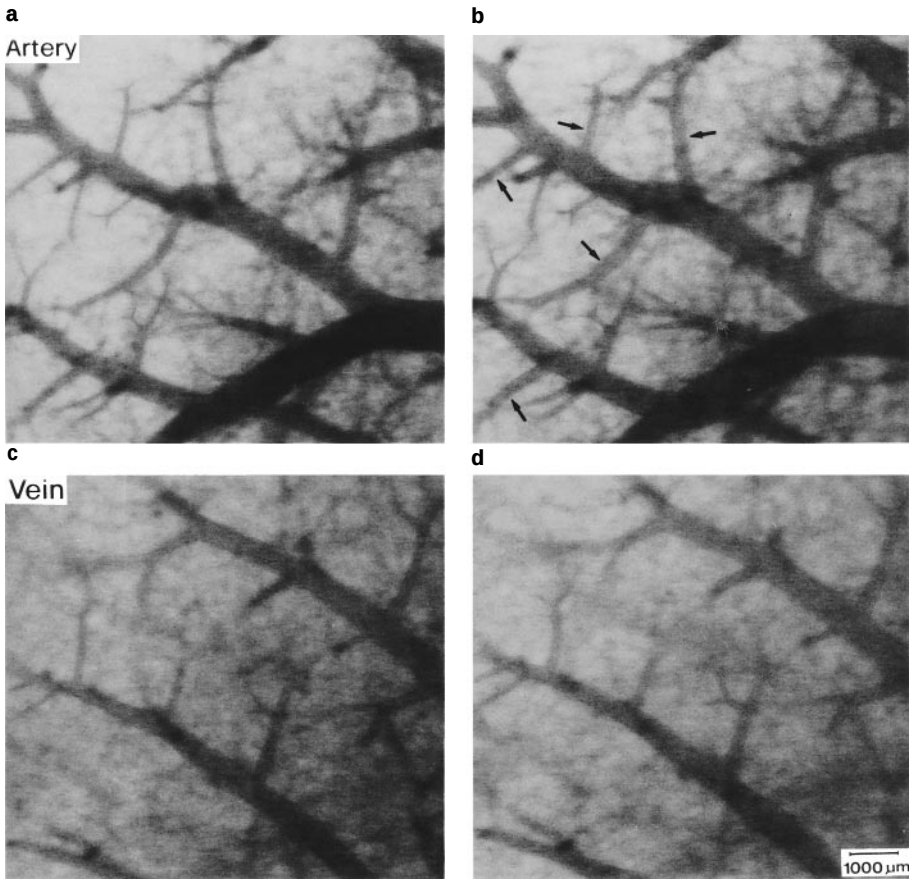

Figure 1 Typical angiograms of small pulmonary arteries (a, b) and veins (c, d) obtained before (a, c) and after (b, d) injection of adrenomedullin (AM, 0.1 nmol kg^{-1}) in the same cat. Solid arrows indicate clear vasodilatations in smaller branches.

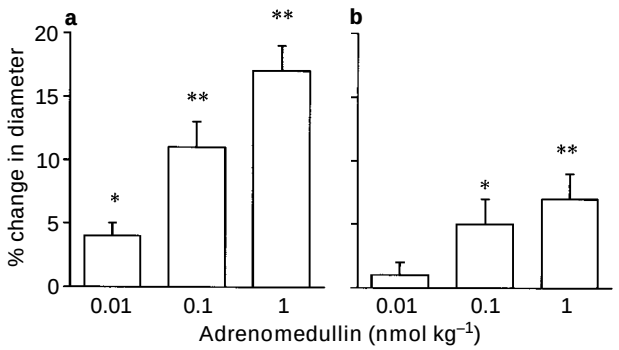

Figure 2 Dose-response effects of adrenomedullin on internal diameter (i.d.) of 100–1100 μm pulmonary arteries (a) and veins (b) under control conditions. The mean vessel sizes of arteries and veins studied for these data were 485 ± 18 and 497 ± 19 μm, respectively. Values are mean $\pm$ s.e.mean of 6 cats. Significant i.d. increase compared to baseline value: $^{*}P<0.05$; $^{**}P<0.01$.

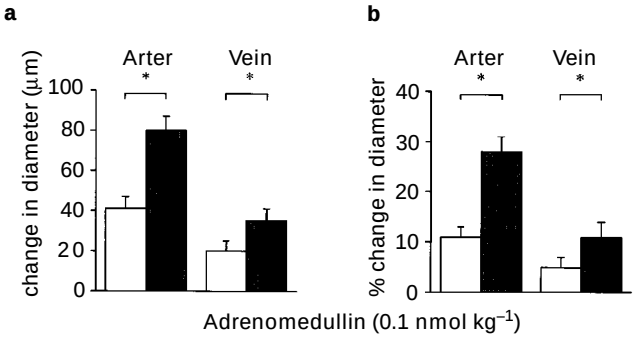

Figure 3 Mean i.d. change (a) and mean percentage change in i.d. (b) for 100–1100 μm vessels in response to an injection of adrenomedullin (0.1 nmol kg^{-1}) were compared between control conditions (open columns) and conditions of hypoxic vasoconstriction (solid columns). Values are mean $\pm$ s.e.mean of 6 cats. The size of vessels studied for these data was the same as that in Figure 2. $^{*}P<0.05$; $^{**}P<0.01$.

0.01 nmol kg^{-1} dose. A comparison of the CGRP- and AM-induced i.d. increases at the same dose showed that the arterial and venous i.d. increases were similar.

The percentage increases of i.d. at different serial segments of the small arteries and veins, were compared between the AM 0.1 nmol kg^{-1} and CGRP 0.1 nmol kg^{-1} (Figure 4). With AM, the i.d. increase of the smaller arteries (100–500 μm) was greater than that of the larger arteries (500–1100 μm). In contrast, CGRP caused a greater increase in the larger arteries (700–1100 μm) than in the smaller arteries (100–500 μm). On the other hand, the venous i.d. responses to the same agents were similar and relatively uniform within serially connected segments. With 0.01 and 1 nmol kg^{-1} AM and CGRP, similar i.d. response patterns to those described above were induced. Those data indicated that AM and CGRP caused quite different i.d. response patterns in the small pulmonary arteries.

Effects of $CGRP_{8-37}$ on AM-induced i.d. increases

The i.d. percentage changes in response to 0.1 nmol kg^{-1} AM under control conditions (Figure 4) and after $CGRP_{8-37}$ pretreatment were compared at different serial segments of the arteries and veins (Figure 5). There was no significant differ-

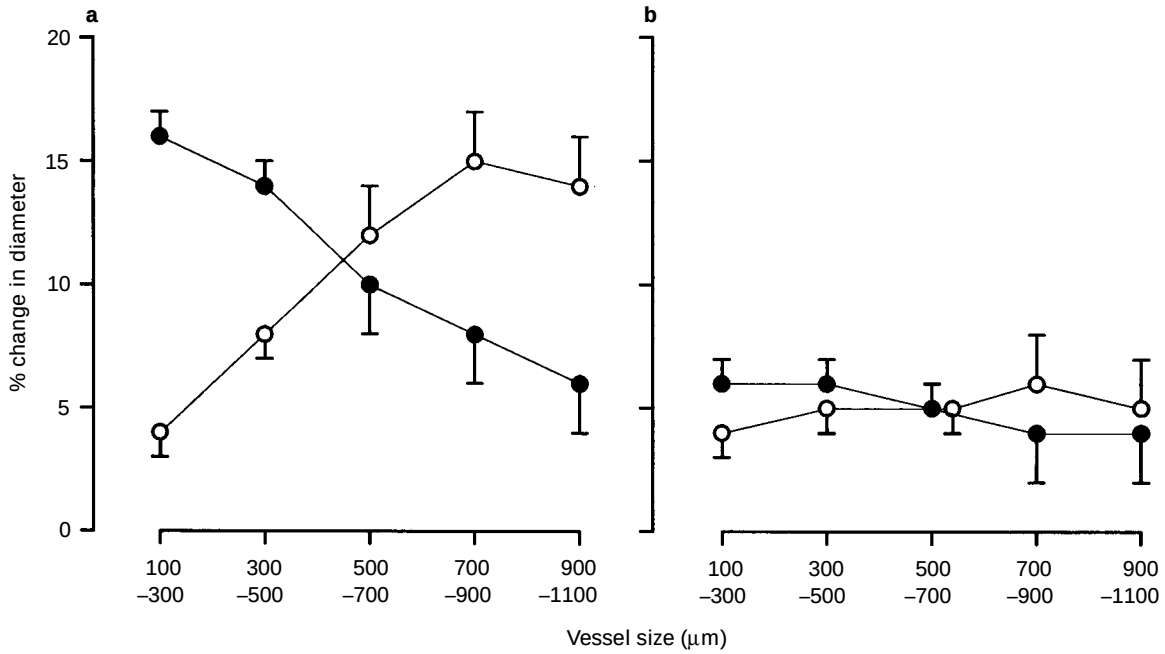

Figure 4 Relationship between vessel size and percentage change in i.d. was compared between (●) adrenomedullin (0.1 nmol kg^{-1}) and (○) CGRP (0.1 nmol kg^{-1}). The i.d. response patterns caused by the two peptides were quite different in arteries (a) but not in the veins (b). Values are mean and vertical lines s.e.mean of 5–6 cats.

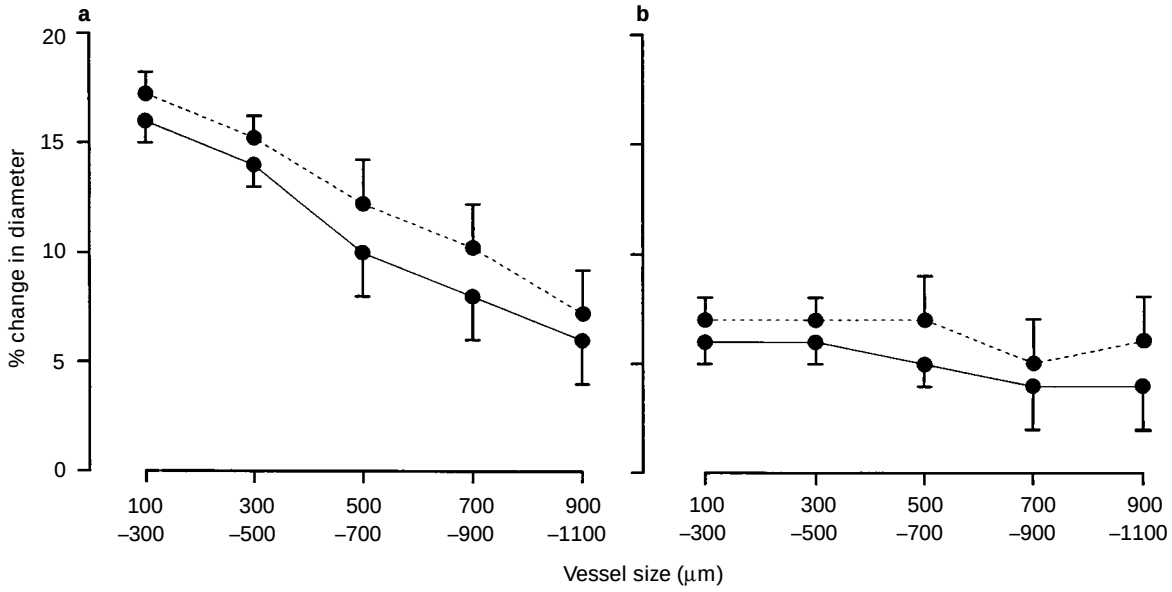

Figure 5 The percentage changes in i.d. in response to 0.1 nmol kg^{-1} adrenomedullin (AM) before (●—●) and after (●- - - -●) $CGRP_{8-37}$ pretreatment are compared at different serial segments of (a) arteries and (b) veins. No significant difference was found between the i.d. responses to AM and AM + $CGRP_{8-37}$ at any of the vascular segments. The values are mean and vertical lines s.e.mean of 5–6 cats.

ence between the two conditions at any of the arterial and venous segments. This indicated that the CGRP antagonist had no effect on the AM-induced i.d. increases in these vessels.

Effects of ganglion blockade on AM- and CGRP-induced i.d. increases

The i.d. changes in response to 0.1 nmol kg^{-1} AM at different serial segments of the arteries and veins in control conditions (Figure 4) were compared with those after hexamethonium bromide pretreatment. There was no significant difference between the two conditions at any of the arterial and venous segments. The i.d. increases in response to 0.1 nmol kg^{-1} CGRP, just as in the case of AM, were unaffected by hexamethonium bromide.

Discussion

The present study shows that intrapulmonary injections of AM induced dose-related increases in i.d. of both small pulmonary arteries and veins (100–1100 μm i.d.), with maximum increases being in 100–500 μm arteries. The i.d. increases in the small vessels were enhanced when their baseline i.d. had been decreased with alveolar hypoxia. In addition, the data demonstrate that the AM-induced i.d. response pattern was quite

different from the CGRP response pattern in the serially connected small pulmonary arteries and that AM-induced i.d. increases were unaffected by $CGRP_{8-37}$ and hexamethonium bromide in all vascular segments studied.

Site of action of AM in the pulmonary vascular bed

The pulmonary vasodilator effect of AM has been studied by measuring pressure-flow relationships. By use of a pump perfusion technique on a haemodynamically separated feline lobar artery at constant flow, it has been shown that intralobar AM injections cause dose-related decreases in pulmonary arterial pressure under conditions of high pulmonary vascular tone (Lippton *et al*., 1994). This was confirmed in rat isolated perfused lungs (Heaton *et al*., 1995; Zhao *et al*., 1996). Such data suggest that AM has vasodilator activity in the pulmonary vascular bed under conditions of high vascular tone. However, the question as to whether the vasodilator response to AM in the serially-connected pulmonary arteries and veins is uniform or nonuniform and which pulmonary vascular segment most strongly contributes to the decrease in pulmonary arterial pressure, have not been answered sufficiently by the indirect methods.

In the present study, we considered the change in i.d. to be better explained as an index of local vasomotor response in a given vessel than the change in calculated resistance estimated from the pressure-flow relationship. Therefore, using an X-ray TV system (Sada *et al*., 1985), we measured i.d. changes of the pulmonary vessels directly. In addition, attention was focused on the small muscular pulmonary arteries and veins on account of their thicker smooth muscle layers compared with pulmonary vessels of other sizes (Sobin *et al*., 1966). They are also highly responsive to many neurohumoral stimuli (Shirai *et al*., 1993; 1994a,b; 1996). The present results indicate that AM injections dilate small arteries non-uniformly and small veins uniformly. Vasodilatation was greater in the arterial side than in the venous side (Figure 2), the maximum dilatation occurring in the more peripheral 100–500 μm arteries (Figure 4).

Changes in the i.d. of the pulmonary arterioles and venules were not observed in this study, but we believe that these vessels are probably far less responsive to AM. The reasons behind this are, (1) that the arterioles and venules have few smooth muscle layers (Sobin *et al*., 1966), and (2) that feline arterioles of less than 100 μm i.d. display only very small i.d. dilatations in response to potent pulmonary vasodilators (such as nitric oxide and isoprenaline) in contrast to larger dilatations in small arteries larger than 100 μm i.d. (Shirai *et al*., 1976). From the previous and present data, we suggest that the 100–500 μm arteries are the most responsive to AM; these vessels contribute most to the AM-induced pulmonary depressor effect.

Possible mechanisms responsible for non-uniform vasodilatations induced by AM injection

The reasons for the greater vasodilator effect of AM on the smaller pulmonary arteries (100–500 μm) than larger ones (500–1100 μm) remains to be clarified. CGRP in the present study and atrial natriuretic peptide (ANP) in a previous study (Shirai *et al*., 1993) dilated the larger pulmonary arteries more than smaller arteries. Therefore, the vasodilator response pattern with AM cannot be ascribed to differences in relaxant ability of vascular smooth muscle between the larger and smaller pulmonary arteries. One of the reasons may be a difference in AM receptor numbers.

Extraction of circulating AM during its passage across the lung has been described by Nishikimi *et al*. (1994) and Yoshibayashi *et al*. (1994). It is therefore conceivable that, in our preparations, the blood concentration of exogenous AM on the venous side was lower than on the arterial side of the pulmonary circulation. This factor may have contributed in part to the present results showing that the arterial response to AM was greater than venous response. However, the higher dose of AM we used would have provided circulating AM levels far greater than the physiological range (Kitamura *et al*., 1994; Ishimitsu *et al*., 1994); indeed, it could have caused great SAP reductions after lung passage (Table 1). We therefore considered AM receptors in the small pulmonary veins to be extensively activated after AM injections.

The contribution of pressure- and flow-sensing mechanisms (Bevan & Laher, 1991) to the AM-induced i.d. response pattern has been discussed. PAP was decreased by ~0.2–1.0 mmHg with AM injections, while LAP and airway pressure remained unchanged (Table 1). Pulmonary blood flow was not measured in this study, but no significant change in cardiac output after AM injection has been obtained in anaesthetized cats (Hao *et al*., 1994). On the other hand, we have previously shown that a mechanically induced ~4 mmHg PAP change and ~30% flow velocity change have no significant effect on the i.d. of small pulmonary vessels (Shirai *et al*., 1994b). Moreover, as described in Methods, the present i.d. response pattern recorded 25–30 s after the AM injection had ended was very similar to that recorded ~10–15 s after AM injection, a stage at which the pressure- and flow-mediated vasomotor responses can be considered to be barely established (Kontos *et al*., 1978; Borgstrom *et al*., 1981; Smiesko *et al*., 1989; Koller & Kaley, 1990) and, therefore, only make a minor contribution to AM-induced i.d. changes. On consideration of these data together, there is only a small possibility that the pressure- and flow-sensing mechanisms contributed to the present i.d. response pattern significantly.

Arterial baroceptor reflex changes caused by systemic hypotension (Table 1) following AM injections may have influenced the i.d. response pattern through an activation of pulmonary sympathetic nerve activity and/or an increase in plasma catecholamine concentration. However, ganglion blockade had no significant effect on the AM-induced i.d. increase. This suggests that the baroceptor-mediated effect is minor.

Possible role of tone-dependent vasodilator effects of AM

Hypoxic pulmonary vasoconstriction (HPV) occurs locally in ~100–700 μm pulmonary arteries and veins, the maximum vasoconstriction occurring in the 200–300 μm arteries (Shirai *et al*., 1994a). This response plays a significant role in most forms of pulmonary hypertension (Marshall, 1990; Weir & Archer, 1995). The present study has shown that AM chiefly dilates the smaller pulmonary arteries (100–500 μm) (Figure 4), which are the main sites for HPV, and that the vasodilator activity of AM is enhanced during HPV in both arteries and veins (Figure 3). On the other hand, a previous study has shown that the plasma level of AM immunoreactivity increases in patients with pulmonary hypertension, in proportion to their increased PAP (Yoshibayashi *et al*., 1994). This result was confirmed in experimental pulmonary hypertensive rats (Shimokubo *et al*., 1995). Moreover, an increase in lung AM binding sites in rats with hypoxia-induced pulmonary hypertension (Zhao *et al*., 1996) has been shown, as well as an increase in lung AM uptake in patients with pulmonary hypertension (Yoshibayashi *et al*., 1994). On consideration of these results together, it is possible that increased plasma AM during pulmonary hypertension may act chiefly on the smaller pulmonary vessels to attenuate the level of pulmonary vascular tone and, in turn, serve to reduce the right ventricular afterload. It has also been suggested that ANP attenuates pulmonary vascular tone with increases in its plasma concentration and in vascular responsiveness to the peptide during pulmonary hypertension (Shirai *et al*., 1993; Perreault & Gutkowska, 1995). However, it should be noted that the main sites of action of ANP (Shirai *et al*., 1993) are different from those for AM; namely the larger pulmonary arteries, but not smaller arteries.

AM vasodilator effects and CGRP receptors

For the systemic circulation, most of the studies into the possible role of CGRP receptors in AM-induced vasodilator effects have been made in the rat. The effect of AM was shown to be inhibited by $CGRP_{8-37}$ in the isolated perfused mesenteric vascular bed (Nuki *et al*., 1993), renal vascular bed (Haynes & Cooper, 1995) and coronary vascular bed (Entzeroth *et al*., 1994) and in the skin microvasculature under anaesthesia (Hall *et al*., 1995). However, no evidence of inhibitory effects was found in the mesenteric, renal, and hindquarters vascular beds of conscious rats (Gardiner *et al*., 1995). Moreover, hypotensive responses to AM were not blocked by $CGRP_{8-37}$ in anaesthetized (Haynes & Cooper, 1995) and conscious animals (Gardiner *et al*., 1995). These studies therefore suggest that, in the systemic circulation, AM can activate CGRP receptors, and can also recognize additional receptors.

The effects of $CGRP_{8-37}$ on AM-induced pulmonary vasodilatations have only been examined *in vitro* in the rat lung. Some investigators have obtained no significant effect of this antagonist on the pulmonary depressor effects of AM (Heaton *et al*., 1995), whereas others have found a significant effect (Zhao *et al*., 1996). The present study conducted on the intact lung of the cat showed that $CGRP_{8-37}$ has no significant effect on AM-induced i.d. increases in any serial segments of the small pulmonary arteries and veins (Figure 5), which suggests that AM dilates both small vessels via $CGRP_{8-37}$-insensitive receptors.

The pulmonary depressor effects of AM and CGRP have been compared in the cat perfused lung lobe (DeWitt *et al*., 1994), but no large difference in the depressor potency between the two peptides was found. In the present study, the mean value of i.d. increases for all 100–1100 μm pulmonary vessels were similar for AM- and CGRP-induced responses consistent with previous results. However, the i.d. response pattern in the serially connected pulmonary arteries was quite different between the two peptides (Figure 4). After CGRP injections, only a small PAP reduction was induced (Table 1) and no significant change in cardiac output was obtained (Andersson, 1989). Moreover, since CGRP-induced i.d. increases were unaffected by hexamethonium bromide, the possibility that the pressure- and flow-sensing mechanisms and the baroreflex mechanisms participate in the CGRP i.d. response pattern is very small, just as in the case of AM as discussed above. Therefore, the difference in response pattern between the peptides cannot be ascribed to these mechanisms. It is most likely that AM acts on receptors other than CGRP receptors to induce the vasodilatation pattern different from that of CGRP. It is noteworthy that chemical cross-linking studies have revealed distinct binding sites for AM in the rat lung with a higher molecular weight than those for CGRP (Owji *et al*., 1995). These authors have also shown that ^{125}I-labelled AM has little affinity at CGRP binding sites.

In conclusion, in the cat, AM induced greater vasodilatation in small pulmonary arteries and lesser vasodilatation in small veins, the maximum dilatation occurring in the more peripheral arterial segments (100–500 μm i.d.). The vasodilator action of AM was potentiated when vascular tone had been increased with alveolar hypoxia. In addition, the AM-induced vasodilator pattern of the arteries was quite different from that for CGRP, and the arterial and venous vasodilatations causesd by AM were unaffected by $CGRP_{8-37}$, suggesting that AM dilated the small pulmonary vessels via its specific receptors and not via CGRP receptors.

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