Analysis of responses to adrenomedullin-(13—52) in the pulmonary vascular bed of rats

BULENT GUMUSEL,1–3 QUINGZHONG HAO,2 ALBERT L. HYMAN,3,4 PHILIP J. KADOWITZ,3,4 HUNTER C. CHAMPION,4 JAW K. CHANG,5 JAWAHAR L. MEHTA,6 AND HOWARD LIPPTON2,7

1Department of Pharmacology, Hacettepe University, Sihhiye, Ankara 06100, Turkey; 2H. L. Laboratories, Incorporated, 3Department of Surgery, Cardiopulmonary Research Lab and 4Department of Pharmacology, Tulane University School of Medicine, and 5Department of Pharmacology, Louisiana State University School of Medicine, New Orleans, Louisiana 70112; 6Phoenix Pharmaceuticals, Incorporated, Mountain View, California 94043; and 7Department of Medicine, University of Florida College of Medicine, Gainesville, Florida 32610

Gumusel, Bulent, Quingzhong Hao, Albert L. Hyman, Philip J. Kadowitz, Hunter C. Champion, Jaw K. Chang, Jawahar L. Mehta, and Howard Lippton. Analysis of responses to adrenomedullin-(13—52) in the pulmonary vascular bed of rats. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1255–H1263, 1998.—The effects of human adrenomedullin-(13—52) [hADM-(13—52)] were investigated in the rat pulmonary vascular bed and in isolated rings from the rat pulmonary artery (PA). Under conditions of controlled blood flow and constant left atrial pressure when tone was increased with U-46619, injections of hADM-(13—52) produced dose-related decreases in lobar arterial pressure. Pulmonary vasodilator responses in the intact rat and vasorelaxant responses to hADM-(13—52) in rat PA rings were inhibited by N5-nitro-L-arginine methyl ester (L-NAME) and L-N3-(1-iminoethyl)-ornithine hydrochloride (L-NIO). Vasorelaxant responses to hADM-(13—52) were also inhibited by methylene blue, endothelium removal, hADM-(26—52), and iberiotoxin, whereas mephenylamine, calcitonin gene-related peptide-(8—37) [CGRP-(8—37)], glibenclamide, and apamin were without effect. Because vasorelaxant responses to NS-1619, a large-conductance Ca2+-activated K+ channel agonist, were not altered by L-NAME and vasorelaxant responses to acetylcholine and CGRP were not altered by hADM-(26—52), the present data suggest that ADM-(13—52) acts on a receptor in the pulmonary vascular bed that is coupled to endothelial nitric oxide release. These data suggest that this nitric oxide release may lead to guanosine 3',5'-cyclic monophosphate accumulation in cultured rat aortic vascular smooth muscle cells (9). The ability of a naturally occurring truncated form of ADM to inhibit the vasodilator response to ADM has not been reported. Furthermore, the mechanism by which ADM induces vasodilatation in the pulmonary vascular bed in the intact rat is uncertain.

METHODS

In vivo. Male Charles River rats (260–340 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg) and allowed to breathe air enriched with oxygen through an endotracheal tube inserted by tracheotomy. The anesthetized animals were strapped in a supine position to a fluoroscopic table, and catheters were inserted into the femoral blood vessels. A specially designed triple-lumen balloon perfusion catheter was constructed (Nu-Med, Hopkinston, NY). This catheter is 245 mm in length, 1.1 mm in OD with a specially curved tip to facilitate passage through the right heart and main pulmonary artery (PA) into the artery supplying the right lower lung lobe. At the distal tip of the catheter is a pressure port through which a 0.25-mm soft-tipped coronary artery angioplasty guide wire is inserted. Two millimeters proximal to this port is a perfusion port that permits easy passage of a 0.34-mm soft-tipped coronary artery guide wire. A plastic nondispensable balloon is affixed to a third port just proximal to the perfusion port.
When fully distended with contrast material, the balloon is 4.0 mm in diameter and 3.5 mm in length. Before introduction, this catheter curve is initially straightened with 0.45 mm straight wire in the pressure port to facilitate passage from the right jugular vein into the right atrium at the tricuspid valve. As the straight wire is removed, the natural curve permits easy entry into the right ventricle. The catheter is then passed over a 0.25-mm soft-tipped guiding catheter to the main PA and then into the right lower lobe artery. Mean pressures in the right lower lobe artery and the aorta were continuously recorded. After intravenous injection of heparin (1,000 U/kg), the balloon is then distended with radiopaque material until the lobar arterial pressure falls to pulmonary capillary wedge pressure. The distal portion of the right lower lung lobe was then perfused with blood removed from a carotid artery with an extracorporeal pump (Masterflex Quick-Load Rotary Pump model 7021–24). The volume of extracorporeal tubing was 1.8 ml. At a perfusion rate of 14 ± 0.6 ml/min, pressure in the perfused lobar artery approximated that in the main PA, and this perfusion rate was taken as control blood flow. Because this catheter perfuses approximately one-sixth of the lung, as determined by measuring lung weight, this perfusion rate approximates physiological flow to the lung sample (i.e., at least 15–20% of the 75–85 ml/min normal total pulmonary blood flow of the rat). After catheterization was completed and constant pulmonary blood flow was established in the right lower lung lobe, pulmonary vasomotor tone was raised by intralobar arterial infusion of U-46619, a thromboxane A₂ mimic, at rates of 1.5–2.5 µg/min. After pressures were stabilized, the intralobar arterial bolus injections of the vasoactive agents were administered. N⁵-nitro-L-arginine methyl ester (L-NAME), L-arginine (L-Arg), sodium nitroprusside (L-NIO), meclofenamate, glibenclamide, and CGRP-(8–37) were administered intravenously over a 15- to 20-min period.

In vitro. Male rats (250–350 g) were anesthetized with pentobarbital sodium (30 mg/kg ip). The rat lungs were quickly removed and immersed in cold (4°C) Krebs-Henseleit (KH) solution (composition in mM: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 25 NaHCO₃, 1.2 MgSO₄, and 10 dextrose). CaCl₂-free solution was made by omitting CaCl₂ from KH solution and by adding 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid to the CaCl₂-free solution. All peptides were added directly to the organ bath in a cumulative concentration manner. The concentrations of all drugs were reported as the final molar concentration in organ chambers.

Drugs and peptides. Peptides [hADM-(1—52), hADM-(13—52), human α-CGRP, rat ADM-(1—50), CGRP-(8—37), and hADM-(26—52)] were provided by Phoenix Laboratories (Mountainview, CA). L-NAME, methylene blue, L-arginine, α-arginine, sodium medofamenate, ACh, α-phenylephrine hydrochloride, glibenclamide, apamin, 3-morpholinosydnonimine hydrochloride (SIN-1 HCl), caffeine, and isoproterenol hydrochloride were obtained from Sigma Chemical (St. Louis, MO). L-NIO was purchased from Alexis (San Diego, CA). IbTX and 1,3-dihydroxy-[2-hydroxyl-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benimidazol-2-one (NS-1619) were provided by Research Biochemicals (Natick, MA). (15S)-hydroxyl-11α,9α (epoxymethano)prosta-5Z,13E-dienic acid (U-46619) was a gift from Upjohn (Kalamazoo, MI). Peptides and all other drugs, except glibenclamide and NS-1619, were dissolved in distilled water. Glibenclamide was dissolved in 2 ml of 0.1 N NaOH and diluted with 5% glucose solution. NS-1619 was dissolved in dimethyl sulfoxide. These stock solutions were diluted with distilled water to desired concentrations. All compounds were added to organ bath medium (15–50 µl); drug concentrations are reported as the final molar concentration in the bath.

Calculations and statistics. Results are expressed as means ± SE. Analysis of variance and Student's unpaired t-test were used where appropriate to assess the significance of differences between means, and P < 0.05 was taken as statistically significant.

RESULTS

Data illustrating the effects of the ADM peptides on the pulmonary vascular bed and on systemic arterial pressure in the intact rat are illustrated in Fig. 1, A and B. Because blood flow to the right lower lung lobe and left atrial pressure were held constant, changes in pulmonary arterial pressure in the right lower lobe directly reflect changes in pulmonary vascular resistance. When arterial pressure in the right lower lung lobe was actively increased by intralobar arterial infusion of U-46619, intralobar bolus injections of hADM-(1—52), hADM-(13—52), and rADM-(1—50) in a similar dose range decreased pulmonary arterial pressure in a dose-dependent manner (Fig. 1A). The pulmonary injections of ADM peptides concurrently decreased systemic arterial pressure in a dose-dependent manner (Fig. 1B). When compared with the ADM peptides studied in the intact rat, human α-CGRP possessed markedly greater pulmonary vasodilator and systemic vasodepressor activity (Fig. 1, A and B).

In another group of experiments under conditions of elevated pulmonary vasomotor tone, vasodilator responses to hADM-(13—52) were compared before and after administration of the nitric oxide synthase inhibitors L-NAME and L-NIO. After administration of L-NAME (100 mg/kg iv) or L-NIO (10 mg/kg iv) and when pulmonary arterial pressure was raised to similar levels with U-46619 (before L-NAME: 35 ± 2 mmHg; after L-NAME: 37 ± 2 mmHg; before L-NIO: control, 36 ± 2 mmHg; after L-NIO: 38 ± 2 mmHg), pulmonary and systemic responses to hADM-(13—52) were significantly decreased (Fig. 2, A and B). After administration of either of these nitric oxide synthesis inhibitors...
inhibitors, the pulmonary vasodilator response to ACh was significantly decreased, whereas the pulmonary vasodilator response to nitroglycerin was significantly increased (Table 1).

Administration of the cyclooxygenase inhibitor meclofenamate (2.5 mg/kg iv), the CGRP receptor antagonist CGRP-(8—37) (10 µg/kg iv), or the K\textsubscript{ATP} channel antagonist glibenclamide (20 mg/kg iv) did not alter pulmonary vasodilator responses to hADM-(13—52) (Table 2). Blockade of CGRP-1 receptors and K\textsubscript{ATP} channels by CGRP-(8—37) and glibenclamide was confirmed by demonstrating inhibition of the pulmonary vasodilator response to CGRP and pinacidil, respectively (Table 1).

The effects of the ADM peptides on isolated rings from the rat PA were investigated, and data illustrating the effects of increasing concentrations of hADM-(13—52) on endothelium-intact and endothelium-denuded rat PA rings are shown in Fig. 3A. When rat PA rings were precontracted by U-46619 (30 nM), hADM-(13—52) (0.3 nM-1 µM) decreased tension in a concentration-dependent manner in endothelium-intact rings. The vasorelaxant response to hADM-(13—52) was significantly attenuated by endothelium removal (Fig. 3A). The vasorelaxant response to isoproterenol was not altered by endothelium removal (data not shown).

Fig. 1. Effects of intralobar arterial bolus injections of human calcitonin gene-related peptide (CGRP), human adrenomedullin (hADM)-(1—52), hADM-(13—52), and rat (r) ADM-(1—50) on pulmonary arterial pressure (A) and systemic arterial pressure (B). Studies were performed in the intact rat when pulmonary vasomotor tone was increased by infusion of U-46619; n, no. of animals.

Fig. 2. Influence of N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME; A) and L-N\textsuperscript{5}-(1-iminoethyl)ornithine hydrochloride (L-NIO; B) administration (10 mg/kg iv) on the pulmonary vasodilator response to hADM-(13—52) in the intact rat; n, no. of animals. *P < 0.05 when compared with corresponding control value.
These results suggest that the method employed to remove the endothelial cell layer did not change to any measurable degree the response to the β-receptor agonist.

The effects of inhibitors of nitric oxide synthesis and soluble guanylate cyclase activation on pulmonary vasorelaxant responses to ADM-(13—52) are illustrated in Fig. 3B. Pretreatment of PA rings with L-NAME (100 µM), L-NIO (30 µM), and methylene blue (10 µM) significantly reduced the pulmonary vasorelaxant response to hADM-(13—52) (Fig. 3B). The inhibitory effect of L-NAME was reversed by addition of excess L-arginine (1 mM) but was not altered by addition of D-arginine (1 mM; Fig. 4). The pulmonary vasorelaxant response to hADM-(13—52) was significantly decreased by hADM-(26—52) (0.1—10 µM; Fig. 5A), whereas meclofenamate (1 µM) and CGRP-(8—37) (1 µM) had no effect (data not shown). hADM-(26—52) reduced the pulmonary vasorelaxant response to hADM-(13—52) in a concentration-dependent manner (Fig. 5A), and this effect could be overcome by increasing concentrations of hADM-(13—52), indicating that hADM-(26—52) acted in a competitive manner. To determine if hADM-(26—52) acts in a selective manner as an ADM receptor antagonist, the effects of hADM-(26—52) on vasorelaxant responses to ACh and human α-CGRP were studied, and pretreatment of rat PA rings with hADM-(26—52) at the highest concentration studied (10 M) did not alter the pulmonary vasorelaxant response.

| Table 1. Influence of L-NAME, L-NIO, CGRP-(8–37), and glibenclamide on pulmonary vasodilator responses to ACh, NTG, CGRP, and pinacidil in the intact rat |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | ACh, µg ia      | NTG (1 µg ia)   | CGRP, µg ia     | Pinacidil (3 µg ia) |
| Control                        | 2.5 ± 0.2       | 4.5 ± 0.3       | 7.6 ± 0.2       |
| L-NAME (100 mg/kg iv)          | 0.4 ± 0.1*      | 0.6 ± 0.1*      | 9.8 ± 0.4*      |
| Control                        | 2.4 ± 0.3       | 4.9 ± 0.4       | 7.8 ± 0.4       |
| L-NIO (10 mg/kg iv)            | 0.5 ± 0.2*      | 0.7 ± 0.2*      | 9.6 ± 0.5*      |
| Control                        | 3.8 ± 0.5       | 7.9 ± 0.7       | 12.1 ± 1.1      |
| CGRP-(8–37) (10 µg/kg iv)      | 1.3 ± 0.4*      | 2.7 ± 0.4*      | 4.1 ± 0.5*      |
| Glibenclamide (10 mg/kg iv)    | 6.8 ± 0.7       | 1.0 ± 0.3*      |

Values are means ± SE and are expressed as a decrease in pulmonary arterial pressure in mmHg; n = 6 rats. L-NAME, N^G-nitro-L-arginine methyl ester; L-NIO, L-N-(1-iminoethyl)ornithine; CGRP, calcitonin gene-related peptide; NTG, nitroglycerin. *P < 0.05 compared with corresponding control.

| Table 2. Influence of medofenate, CGRP-(8–37) and glibenclamide on pulmonary vasodilator responses to hADM-(13–52) in the intact rat |
|---------------------------------|-----------------|-----------------|
|                                | hADM-(13-52), µg ia |
|                                | 1               | 3               | 10              |
| Control                        | 3.6 ± 0.4       | 5.8 ± 0.5       | 8.1 ± 0.7       |
| Medofenate (2.5 mg/kg iv)      | 3.2 ± 0.3       | 5.6 ± 0.4       | 7.9 ± 0.6       |
| Control                        | 3.4 ± 0.3       | 5.7 ± 0.4       | 8.0 ± 0.4       |
| CGRP-(8-37) (10 µg/kg iv)      | 3.1 ± 0.4       | 5.6 ± 0.5       | 7.6 ± 0.5       |
| Control                        | 3.2 ± 0.5       | 5.5 ± 0.6       | 8.2 ± 0.8       |
| Glibenclamide (20 mg/kg iv)    | 3.1 ± 0.4       | 5.1 ± 0.7       | 8.0 ± 0.6       |

Values are means ± SE and are expressed as a decrease in pulmonary arterial pressure in mmHg; n = 6 rats. hADM, human adrenomedullin.

Fig. 3A: influence of endothelial (E) cell removal on the pulmonary vasorelaxant response to hADM-(13—52) on rat pulmonary arterial (PA) rings precontracted with U-46619. B: influence of L-NAME, L-NIO, and methylene blue on the pulmonary vasorelaxant response to hADM-(13—52) in precontracted rat PA rings; n, no. of animals. *P < 0.05 when compared with corresponding control value.
response to ACh or human α-CGRP (data not shown). Moreover, hADM-(26—52) (10 µM) had no direct contractile or vasorelaxant activity in PA rings. In contrast, CGRP-(8—37) (1 µM) significantly decreased the vasorelaxant response to human α-CGRP (data not shown).

To determine if a mechanism in addition to an endothelium-derived relaxing factor (EDRF)-dependent pathway mediates the pulmonary vasorelaxant response to ADM, experiments were performed using inhibitors of K⁺ channel mechanisms involved in vasodilation, and these results are illustrated in Fig. 5B. When rat PA rings with intact endothelial cell layers were precontracted with U-46619, prior treatment with IbTX (100 nM) significantly decreased the vasorelaxant response to hADM-(13—52), whereas glibenclamide (1 µM) and apamin (1 µM) did not alter the vasorelaxant response to hADM-(13—52) (Fig. 5B). To determine if responses to a nonpeptide EDRF-dependent vasorelaxant (ACh), a substance that directly releases nitric oxide (SN-1), and a putative activator of large-conductance Ca²⁺-dependent K⁺ channels (NS-1619) are influenced by these K⁺ channel blockers, additional experiments were performed, and these results are illustrated in Fig. 6, A–C. When rat PA rings with intact endothelium were precontracted with U-46619, ACh, SN-1, and NS-1619 decreased tension in a concentration-dependent manner (Fig. 6, A–C). The vasorelaxant responses to ACh, SN-1, and NS-1619 were significantly decreased by pretreatment with IbTX, whereas pretreatment with glibenclamide and apamin did not alter the pulmonary vasorelaxant response to ACh, SN-1, and NS-1619 (Fig. 6, A–C). Moreover, the pulmonary vasorelaxant response to NS-1619 was not altered by L-NAME (data not shown).

Additional experiments were performed to determine if precontraction by voltage-mediated and receptor-mediated mechanisms differentially influences the pulmonary vasorelaxant response to hADM-(13—52). Data showing the effects of hADM-(13—52) on rat PA rings precontracted with U-46619, phenylephrine, and varying concentrations of KCl are illustrated in Fig. 7. Vasorelaxant responses to hADM-(13—52) on rat PA rings precontracted with U-46619 and phenylephrine were similar. In contrast, the vasorelaxant response to hADM-(13—52) on rat PA rings precontracted with KCl (30 mM) was significantly reduced when compared with responses in PA rings precontracted by U-46619 or phenylephrine (Fig. 7). Moreover, the pulmonary vasorelaxant response to hADM-(13—52) was abolished in rat PA rings precontracted with 40 and 60 mM KCl.
To determine if ADM-(13—52) possesses the ability to influence voltage-mediated and receptor-mediated responses, additional experiments were performed, and these results show that the contractile response to KCl was not altered by pretreatment with varying concentrations of hADM-(13—52) (data not shown). In contrast, pretreatment with hADM-(13—52) inhibited the contractile response to U-46619 and phenylephrine in a concentration-dependent manner (data not shown). The present data indicate that hADM-(13—52) has little or no effect on voltage-mediated contraction but possesses the ability to prevent and reverse receptor-mediated contractile responses in rat PA rings.

To determine if hADM-(13—52) influences Ca\(^{2+}\) influx, additional experiments were performed and show that, after removal of external Ca\(^{2+}\) in the organ bath, a single concentration of U-46619 (0.1 µM), phenylephrine (1 µM), and caffeine (10 mM) produced transient contraction in rat PA rings that may reflect the amount of Ca\(^{2+}\) present in the intracellular stores necessary to produce contraction. The pulmonary contractile response to all three vasoconstrictor substances studied was not altered by hADM-(13—52) (0.03, 0.1, 0.3 µM; data not shown). To determine if hADM-(13—52) acts as a Ca\(^{2+}\) channel blocking agent to alter vascular contraction, additional experiments were performed in which maximal depolarization by KCl of rat PA rings with subsequent addition of CaCl\(_2\) to contract the rings was induced before addition of hADM-(13—52). Subsequent addition of hADM-(13—52) to these same rat PA rings produced a small, transient pulmonary vasorelaxant response; however, there was no significant reduction in baseline tension in rat PA rings (Fig. 8).

Fig. 6. Influence of IbTX, glibenclamide, and apamin on the pulmonary vasorelaxant response to NS-1619 (A), SIN-1 (B), and ACh (C) in precontracted rat PA rings; n, no. of animals. *P < 0.05 when compared with corresponding control value.

Fig. 7. Effects of precontractions by U-46619, phenylephrine, and varying concentrations of KCl on the pulmonary vasorelaxant response to hADM-(13—52) in rat PA rings; n, no. of animals. *P < 0.05 when compared with corresponding control value.
Addition of verapamil, unlike hADM-(13—52), to rat PA rings under similar conditions but without addition of hADM-(13—52) produced 100% vascular relaxation (Fig. 8B).

DISCUSSION

Results of the present study demonstrate that hADM-(13—52), hADM-(1—52), and rat ADM-(1—50) possess similar pulmonary vasodilator activity in the pulmonary vascular bed of the rat in vivo when tone was increased with U-46619. Because pulmonary blood flow and left atrial pressure were held constant, changes in pulmonary arterial pressure directly reflect changes in pulmonary vascular resistance. Pulmonary vasodilator and systemic vasodepressor responses to hADM-(13—52) and ACh in vivo were markedly inhibited by L-NAME and L-NIO, whereas the vasodilator response to nitroglycerin was enhanced. L-NAME and L-NIO have been reported to increase the vasorelaxant activity of nitroglycerin (35). The pulmonary vasodilator response to hADM-(13—52) was not altered by meclofenamate, CGRP-(8—37), or glibenclamide, suggesting that the present data in vivo are consistent with previous in vitro data from this laboratory and indicate that the pulmonary vasodilator response to hADM-(13—52) is not mediated by cyclooxygenase products, activation of CGRP-1 receptors, or K_{ATP} channels (13).

The present data demonstrate that the vasorelaxant response to hADM-(13—52) on rat PA rings was inhibited by endothelium removal, L-NAME, L-NIO, and methylene blue and that the pulmonary vasorelaxant response to hADM-(13—52) was not altered by meclofenamate, CGRP-(8—37), and glibenclamide. Moreover, hADM-(26—52) acted in a selective manner, since vasorelaxant responses to hADM-(13—52) were attenuated without altering responses to ACh and CGRP. Because the inhibitory effects of L-NAME and L-NIO were prevented by L-arginine but not by D-arginine, these results indicate that ADM-(13—52) relaxes rat PA rings by releasing nitric oxide from the endothelium. The present data in the intact rat are consistent with the data in vitro on rat PA rings, suggesting that hADM-(13—52) dilates the pulmonary vascular bed of the rat by activating ADM receptors on the endothelium and promoting the release of nitric oxide. Moreover, the present data in the intact rat are also consistent with data in rat PA rings, suggesting that ADM-(13—52) dilates the rat pulmonary vascular bed by a mechanism independent of the formation of cyclooxygenase products, activation of CGRP-1 receptors, or K_{ATP} channels. CGRP receptors have been reported to mediate the peripheral vasorelaxant response to ADM in vitro (37, 43), and it has been suggested ADM directly dilates vascular smooth muscle cells through activation of CGRP receptors (43). The present data indicate that ADM-(13—52) does not directly relax rat pulmonary vascular smooth muscle. In addition, the present data indicate that ADM-(13—52) dilates the pulmonary vascular bed in vivo independent of activation of a “CGRP-like” receptor (43).

In rat PA rings, vasorelaxant responses to hADM-(13—52), ACh, SIN-1, and NS-1619 were inhibited by IbTx but not by apamin, suggesting that the vasorelaxant response to hADM-(13—52) is also mediated by BK_{Ca} channels but not SK_{Ca} channels. Because the vasorelaxant response to NS-1619, an agent reported to be a putative BK_{Ca} channel activator (8, 38), was not altered by nitric oxide synthesis inhibitors, the present data suggest that hADM-(13—52) acts on ADM receptors on the endothelium to release nitric oxide. These data suggest that the nitric oxide activates BK_{Ca} channels with subsequent activation of soluble guanylate cyclase to raise guanosine 3',5'-cyclic monophosphate (cGMP) levels. The present results may suggest the presence of hADM-(13—52) receptors in the rat pulmonary vascular bed that are coupled to endothelial-derived nitric oxide release and cGMP-dependent K^{+} channel activation, which induces a vasorelaxant response by hyperpolarizing vascular smooth muscle cells. Although a pathway for cGMP-dependent calcium-

Fig. 8. A: typical tracings illustrating the pulmonary vascular contractile responses to phenylephrine (0.3 μM; a), phenylephrine (1 μM) in Ca^{2+}-free solution (b), and phenylephrine in Ca^{2+}-free solution 15 min after pretreatment with hADM-(13—52) (0.3 μM; c). B: typical tracings illustrating the differential influence of hADM-(13—52) (0.3 μM; a) and verapamil (0.1 μM; b) on the pulmonary vascular contractile response to a maximal depolarizing concentration (118 mM) followed by addition of CaCl_{2} (2.5 mM).
activated potassium channel activation resulting in vasorelaxation has been reported (1, 3, 4, 24, 49), the present data suggest hADM-(13—52) uses this pathway to modulate pulmonary vascular responses. hADM-(13—52) reversed contractile responses to phenylephrine and U-46619 without altering the response to KCl. This differential effect of hADM-(13—52) is likely due to hyperpolarization of vascular smooth muscle. This hypothesis is supported by previous work showing that, when the vascular smooth muscle cell membrane is hyperpolarized, receptor-mediated Ca\textsuperscript{2+} influx is decreased (6, 21, 33). The present data provide support for this hypothesis, since hADM-(13—52) did not alter contractile responses to phenylephrine and caffeine under Ca\textsuperscript{2+}-free conditions. Moreover, hADM-(13—52) may act to inhibit Ca\textsuperscript{2+} influx required for receptor-mediated contraction (6, 21, 48). The degree of vascular hyperpolarization by ADM-(13—52) does not appear to influence contractions due to release of intracellular Ca\textsuperscript{2+} stores (21, 22). The effect of hADM-(13—52) on voltage-mediated and receptor-mediated contraction also appears to be due to the ability of KCl-induced pulmonary contraction to inhibit the modulatory effects of ADM-(13—52). The present data provide support for such a hypothesis, since ADM-(13—52) had little or no pulmonary vasorelaxant activity on PA rings precontracted with KCl. The present data indicate that ADM-(13—52) does not inhibit Ca\textsuperscript{2+} influx by influencing L-type Ca\textsuperscript{2+} channels, since ADM-(13—52), unlike verapamil, did not alter contractions induced by CaCl\textsubscript{2} in maximally depolarized rat PA rings.

Conflicting data exist on the ability of L-NAME to inhibit the pulmonary vasodilator response to endogenous peptides in the in vitro isolated perfused rat lungs, and L-NAME has been reported to have actions in addition to inhibiting nitric oxide synthase (5, 13, 31, 36, 40, 46). In the present study, pulmonary vasodilator responses to hADM-(13—52) were reduced by two chemically dissimilar nitric oxide synthase inhibitors in the intact rat and by these same inhibitors and by methylene blue in PA rings from the rat. These data, along with results showing that vasorelaxant responses to hADM-(13—52) were abolished by endothelial denudation, provide support for the hypothesis that hADM-(13—52) acts by releasing nitric oxide from the endothelium.

The lung expresses ADM receptors on endothelial and vascular smooth muscle cells (20, 23, 39). The reason for the expression of a large number of ADM receptors on vascular smooth muscle cells is unclear but may relate to the ability of ADM to influence vascular function over time (17). Because endothelial cells synthesize ADM and endothelial cells possess ADM receptors, the endothelium may act to regulate the pulmonary circulation by releasing ADM.

In summary, the results of the present study demonstrate that the pulmonary vasodilator response to hADM-(13—52) in the intact rat and the vasorelaxant response to hADM-(13—52) in rat PA rings were inhibited by hADM-(26—52), L-NAME, L-NIO, methylene blue, endothelium removal, and IbTX. Meclofenamate, CGRP-(8—37), glibenclamide, and apamin were without effect on the response to hADM-(13—52). Because the pulmonary vasorelaxant response to the putative BK\textsubscript{Ca} channel opener NS-1619 was not altered by L-NAME and vasorelaxant responses to ACh and CGRP were not altered by hADM-(26—52), the present data suggest that ADM-(13—52) acts on receptors in the pulmonary vascular bed that are coupled to endothelium-derived nitric oxide release and cGMP-dependent K\textsuperscript{+} channel activation, which is associated with membrane hyperpolarization and a pulmonary vasorelaxant response. However, the effects of ADM-(13—52) on smooth muscle cGMP levels and on K\textsuperscript{+} channel activity should be measured in future experiments in rat PA.

Address for reprint requests: A. L. Hyman, Dept. of Pharmacology, Tulane Univ. School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112.

Received 20 September 1996; accepted in final form 23 December 1997.

REFERENCES