British Journal of Pharmacology (1996) 119, 1477-1483

Effects of adrenomedullin and calcitonin gene-related peptide on airway and pulmonary vascular smooth muscle in guinea-pigs

Arthur Pinto, Kiyohisa Sekizawa, Mutsuo Yamaya, Takashi Ohrui, Yu Xia Jia & [1]Hidetada Sasaki

Department of Geriatric Medicine, Tohoku University School of Medicine, Aoba-ku Seiryo-machi 1-1 Sendai 980, Japan

1 The airway and pulmonary vascular effects of adrenomedullin were studied in the guinea-pig isolated trachea, main bronchi and pulmonary artery *in vitro* and compared to the effects of calcitonin gene-related peptide (CGRP).

2 In tracheal rings, CGRP (1 nM to 1 μM) potentiated the cholinergic contractions induced by electrical field stimulation (EFS) at 5 Hz in a concentration-dependent manner. At a concentration of 1 μM, CGRP slightly decreased the responses to log EFS frequency, producing 50% of the maximum contraction from a control value of 0.77 ± 0.10 Hz to 0.54 ± 0.05 Hz without a significant effect on the concentration-response curves to acetylcholine (ACh). In contrast, adrenomedullin (1 nM to 1 μM) did not alter either EFS-induced cholinergic or ACh-induced contractions.

3 In bronchial strips, CGRP (1 nM to 1 μM) slightly reduced both the non-adrenergic non-cholinergic (NANC) contraction induced by EFS at 10 Hz and the substance P (1 μM)-induced contraction in a concentration-dependent manner, whereas adrenomedullin (1 nM to 1 μM) was without effect.

4 Neither CGRP (1 μM) nor adrenomedullin (1 μM) altered NANC relaxation induced by EFS at 5 Hz in tracheal rings precontracted with histamine (10 μM).

5 Adrenomedullin (1 nM to 1 μM) and CGRP (1 nM to 1 μM) induced a concentration-dependent relaxation of the histamine (10 μM)- and prostaglandin $F_{2\alpha}$ (10 μM)-precontracted pulmonary arterial rings with intact endothelium with a similar potency.

6 Neither removal of the endothelium nor N^G-nitro-L-arginine methyl ester (100 μM) altered the vasorelaxant effects of adrenomedullin (1 nM to 1 μM) and CGRP (1 nM to 1 μM).

7 The putative CGRP receptor antagonist, $CGRP_{8-37}$ (1 μM to 10 μM) concentration-dependently attenuated the CGRP (3 nM to 30 nM)-induced vasorelaxant actions, whereas it had no effect on the relaxation of vessel rings induced by adrenomedullin (3 nM to 30 nM).

8 These results suggest that adrenomedullin is a potent vasodilator of the pulmonary artery without any bronchomotor effect in the guinea-pig lung, and that the vasorelaxant actions of adrenomedullin are not mediated via the activation of $CGRP_1$ receptors.

Keywords: Airway smooth muscle; pulmonary artery; endothelium; adrenomedullin; calcitonin gene-related peptide

Introduction

Adrenomedullin, a peptide from human phaeochromocytoma tissue, consists of 52 amino acids and shares slight homology with calcitonin gene-related peptide (CGRP) (Kitamura *et al.*, 1993a). Studies in excised human tissue using an antibody to adrenomedullin have shown that the lung contains the largest amount of adrenomedullin, with smaller quantities in the kidney, adrenal gland, and plasma (Kitamura *et al.*, 1993a). RNA blot analysis indicates that rat adrenomedullin mRNA is expressed in the adrenal glands, lung, heart, kidney, spleen, duodenum, and submandibular glands (Kitamura *et al.*, 1993b; Perret *et al.*, 1993). Adrenomedullin has potent systemic (Ishiyama *et al.*, 1993; Hao *et al.*, 1994) and pulmonary (Heaton *et al.*, 1995) vasodilator activity. Although the airway and pulmonary vascular effects of CGRP have been well studied (Barnes *et al.*, 1991), the actions of adrenomedullin in the lung are less clear. The role of adrenomedullin in the lung and its effect on bronchomotor tone are essentially unknown.

We have therefore investigated the effects of adrenomedullin on cholinergic and non-adrenergic non-cholinergic (NANC) contractions, and the NANC relaxant response in tracheal and bronchial tissues in guinea-pigs *in vitro*. We have also examined the vasodilator effects of adrenomedullin in pulmonary arteries with and without endothelium, and compared the effects of adrenomedullin to those of CGRP.

[1] Author for correspondence.

Methods

Tissue preparation

Male Hartley guinea-pigs (Funabashi Farm, Shizuoka, Japan), weighing 250-350 g, were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.). Tracheae, main bronchi and pulmonary artery trunks were removed carefully and immersed in Krebs-Henseleit solution, pH 7.4 of the following composition (mM): NaCl 118, KCl 5.9, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.5 and glucose 5.6, equilibrated continuously with 95% O_2 and 5% CO_2. Following careful removal of adherent fat and connective tissue, transverse rings (3 mm long) were cut from the trachea. The two main bronchi were opened longitudinally along the anterior side, and cut into strips. The pulmonary artery was cut into rings of 3 mm width.

The preparations were mounted in organ baths containing 5 ml Krebs-Henseleit solution maintained at 37°C. Isometric tension was continuously measured with a force transducer (TB 612-T, Nihon Koden, Japan) connected to a pen recorder (HORIZ-8K, San-ei Co., Japan). Tissues were equilibrated for 60 min under approximately 0.5 g resting load for the trachea, 0.25 g resting load for the main bronchi and 1 g resting load for the pulmonary artery. The trachea and bronchial tissues were placed between two rectangular platinum electrodes (6×40 mm) for electrical field stimulation (EFS; supramaximal voltage of 50 V and 1.0 ms duration).

Cholinergic contraction in tracheal rings

Before the experiment, capsaicin (10 μM) was introduced and washed out 30 min after the pretreatment to deplete en-

dogenous tachykinins. Tissues were also pretreated with indomethacin (1 μM), phentolamine (10 μM) and propranolol (1 μM) (Sekizawa *et al.*, 1993). For experiments involving cholinergic contractile responses in guinea-pig trachea, stimulation was applied for 30-s periods every 5 min at a frequency of 5 Hz, which caused approximately 50% of the maximal neural contraction response. After at least four stable responses of equal magnitude were obtained, we added increasing concentrations of CGRP (from 1 nM to 1 μM) or adrenomedullin (from 1 nM to 1 μM) and repeated stimulation 5 min after the administration of each concentration of drugs. To determine the effects of CGRP and adrenomedullin on stimulus frequency-response curves we stimulated tissues by varying the stimulus frequencies from 1 Hz to 50 Hz. After the first frequency-response curve was completed, we performed the second one 5 min after the administration of either CGRP or adrenomedullin at a concentration of 1 μM, which was the maximum concentration used in the present study. The contractile responses to EFS obtained under these conditions were completely blocked by both atropine (1 μM) and tetrodotoxin (1 μM), indicating that the responses were caused by stimulation of cholinergic nerves. To determine whether the effects of CGRP and adrenomedullin are mediated by postjunctional cholinergic mechanisms, we studied the effects of CGRP (1 μM) and adrenomedullin (1 μM) on the cumulative concentration-response curves to acetylcholine (from 1 nM to 1 mM).

Excitatory NANC response in bronchial strips

To determine the baseline response, bronchial tissues were stimulated electrically for 30 s periods every 5 min at a frequency of 10 Hz in the presence of atropine (1 μM), indomethacin (1 μM), phentolamine (10 μM) and propranolol (1 μM) (Aikawa *et al.*, 1992). After at least four stable responses of equal magnitude were obtained, we added increasing concentrations of CGRP (from 1 nM to 1 μM) or adrenomedullin (from 1 nM to 1 μM) and repeated the stimulation 5 min after the administration of each concentration of drug. CGRP or adrenomedullin were administered after the preceding contraction had returned to the baseline. The contractile responses induced under these conditions were completely abolished by tetrodotoxin (1 μM), confirming the neural nature of the response.

The effects of CGRP (from 1 nM to 1 μM) and adrenomedullin (from 1 nM to 1 μM) on the contractile responses to exogenous substance P (SP) (1 μM, which produced a contractile response equivalent to that produced by EFS) were also evaluated.

Inhibitory NANC response in tracheal rings

To examine the effects of adrenomedullin and CGRP on NANC-mediated relaxations, tracheal rings were precontracted with submaximal concentration of histamine (10 μM) in the presence of atropine (1 μM), indomethacin (1 μM), phentolamine (10 μM) and propranolol (1 μM). EFS was applied for 60 s period at a frequency of 5 Hz before and 5 min after the administration of either CGRP (1 μM) or adrenomedullin (1 μM). To determine the effects of CGRP and adrenomedullin on NANC relaxant responses, the NANC relaxation in each preparation before the addition of CGRP or adrenomedullin was taken as 100%. The relaxant responses under these conditions were completely abolished by tetrodotoxin (1 μM).

Vasorelaxant response in pulmonary arteries

For each artery, two rings were prepared one of which was gently and repeatedly rubbed on its intimal surface by means of a cotton-tip applicator in order to remove the vascular endothelium. The effectiveness of this manoeuvre was assessed by checking the absence of a vasorelaxant response to acetylcholine (from 10 to 100 μM). Tissues were then washed and left for 30 min in organ baths. Next, a cumulative concentration-contraction curve was constructed for histamine (from 1 μM to 100 μM) after which tissues were washed and left for 30 min before being re-contracted to a single, submaximal concentration (usually 10 μM) of histamine. A cumulative concentration-relaxation curve to either CGRP (from 1 nM to 1 μM) or adrenomedullin (from 1 nM to 1 μM) was obtained after a stable tone had been induced by histamine. To determine whether the effects of adrenomedullin and CGRP are mediated by nitric oxide, tissues with intact endothelium were pretreated with N^G-nitro-L-arginine methyl ester (L-NAME; N^G-nitro-L-arginine 100 μM) 20 min before the administration of histamine (10 μM). We also tested the vasorelaxant effects of CGRP (from 1 nM to 1 μM) and adrenomedullin (from 1 nM to 1 μM) in tissues precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 10 μM).

To determine the effects of the putative CGRP receptor antagonist, $CGRP_{8-37}$ (Chiba *et al.*, 1989) on CGRP- and adrenomedullin-induced relaxations of pulmonary artery, tissues with intact endothelium were pretreated with either a 1 μM or 10 μM concentration of $CGRP_{8-37}$ for 15 min after the administration of histamine (10 μM). The effects of CGRP (from 3 nM to 30 nM) or adrenomedullin (from 3 nM to 30 nM) were then examined cumulatively after a stable tone had been induced by histamine.

The following drugs were used: tetrodotoxin, capsaicin, indomethacin, histamine dihydrochloride, acetylcholine chloride, calcitonin gene-related peptide, calcitonin gene-related $peptide_{8-37}$, N^G-nitro-L-arginine methyl ester (Sigma), phentolamine (Ciba Geigy), prostaglandin $F_{2\alpha}$ (Ono Pharmaceutical Co., Ltd., Tokyo, Japan).

All values are expressed as mean ± s.e. mean. Statistical analysis was performed by one way analysis of variance. Significance was accepted at $P<0.05$.

Results

Cholinergic contraction in tracheal rings

Adrenomedullin up to 1 μM did not change either the baseline tension or the contractile response to EFS at 5 Hz (Figure 1a). Likewise, CGRP up to 1 μM did not alter the baseline tension, but did cause a concentration-dependent increase in the contractile response to EFS at 5 Hz (Figure 1a). EFS frequency-response curves were evaluated by mean (± s.e. mean) log frequency of the stimulation producing 50% of the maximal contraction to EFS (ES_{50}). EFS frequency-response curves were reproducible and ES_{50} did not differ significantly between the first and second frequency-response curves in the absence of drugs (0.76 ± 0.08 Hz vs. 0.75 ± 0.09 Hz; $P>0.50$, $n=7$). Likewise, adrenomedullin (1 μM) did not alter the ES_{50} (0.76 ± 0.09 Hz vs. 0.74 ± 0.08 Hz; $P>0.50$, $n=7$) (Figure 1b). However, CGRP (1 μM) shifted the mean frequency-response curve to the left and significantly reduced ES_{50} (0.77 ± 0.10 Hz vs. 0.54 ± 0.05 Hz; $P<0.05$, $n=7$) (Figure 1c). In contrast to EFS-induced contractions, ACh-induced contractions were not affected by adrenomedullin (1 μM) ($-\log EC_{50} = 5.39 \pm 0.48$ M vs. 5.41 ± 0.40 M; $P>0.50$, $n=7$) or CGRP (1 μM) ($-\log EC_{50} = 5.46 \pm 0.51$ M vs. 5.38 ± 0.50 M; $P>0.30$, $n=7$).

Excitatory NANC response in bronchial strips

EFS produced a long lasting NANC contraction that was reproducible on repeated stimulations within any given tissue (Aikawa *et al.*, 1992). The contractions produced by SP (1 μM) were also reproducible, and were not significantly different in amplitude from those elicited by EFS (182 ± 26 mg vs. 200 ± 29 mg with adrenomedullin; 193 ± 25 mg vs. 178 ± 27 mg with CGRP) ($P>0.20$, $n=7$). Adrenomedullin (1 μM) did not alter the amplitude of either EFS- or SP-induced contractions (Table 1). However, CGRP (1 μM) decreased the amplitude of

both EFS- and SP-induced contractions. The inhibition of both responses was concentration-dependent and the percentage inhibition from baseline did not differ significantly at any concentration (Table 1).

Inhibitory NANC response in tracheal rings

EFS produced a long lasting NANC relaxation in tracheal rings precontracted with histamine (10 μM). Neither CGRP (1 μM) nor adrenomedullin (1 μM) altered the tone raised by histamine. Likewise, NANC relaxant responses were not significantly changed by CGRP (100.4±6.6% of control, $P>0.50$, $n=7$) or adrenomedullin (98.5±7.0% of control, $P>0.30$, $n=7$).

Vasorelaxant response in pulmonary arteries

In rings with intact endothelium, histamine (10 μM) induced the same degree of tone as $PGF_{2\alpha}$ (10 μM) (1.4±0.2 g in histamine vs. 1.4±0.3 g in $PGF_{2\alpha}$; $P>0.50$, $n=7$). Both adreno-

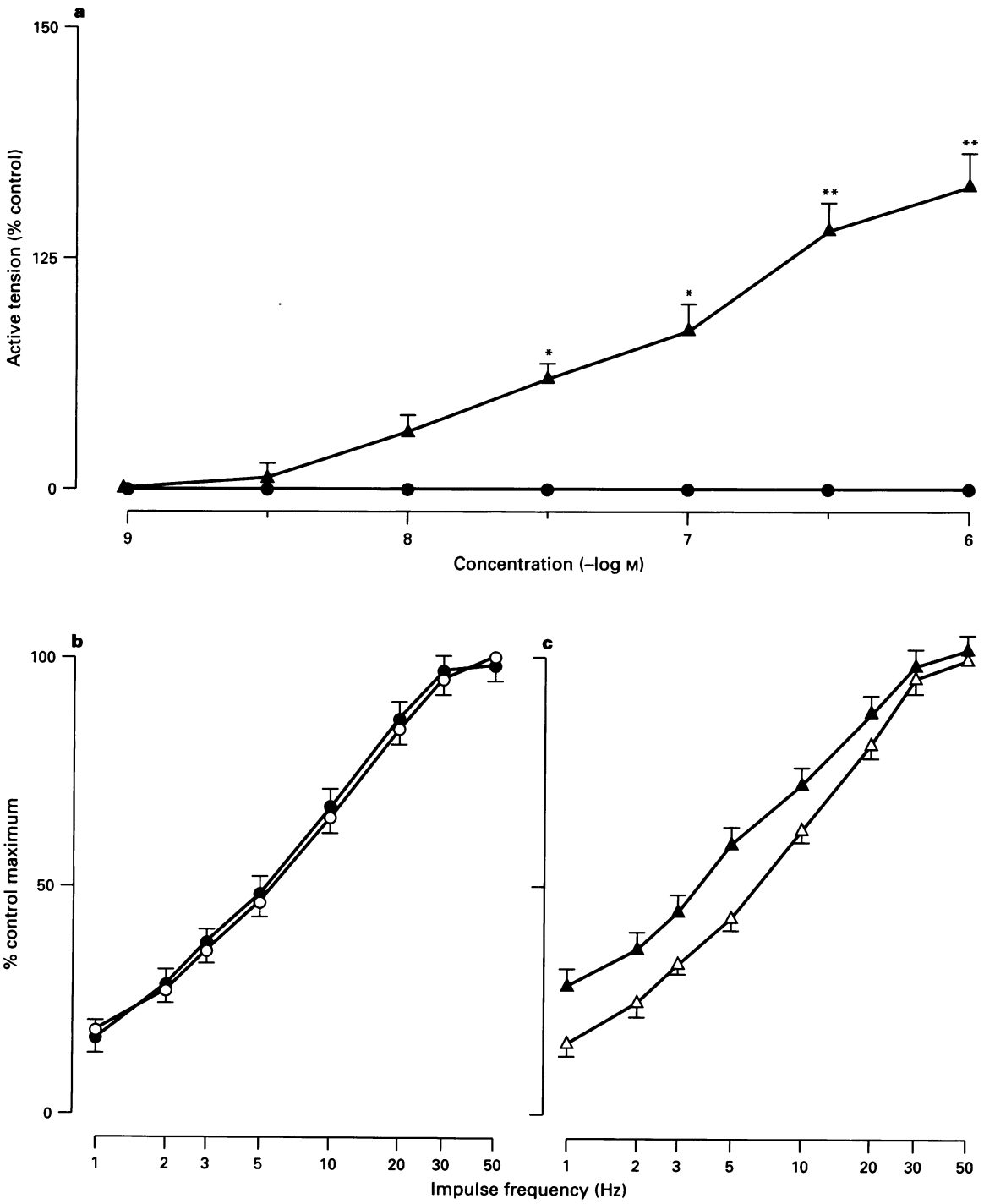

Figure 1 Concentration-response effects of adrenomedullin (●) and calcitonin gene-related peptide (CGRP; ▲) on the contractile response to electrical field stimulation (EFS) at 5 Hz in tracheal rings (a), and frequency-dependent contractile response to EFS in the presence (closed symbols) or absence (open symbols) of adrenomedullin (1 μM) (b) and CGRP (1 μM) (c) in tracheal rings. Data are shown as mean±s.e.mean of values from 7 guinea-pigs. Significant differences from corresponding control values are indicated by $*P<0.05$ and $**P<0.01$.

medullin and CGRP induced a concentration-dependent relaxation of the histamine (Figure 2a)-and $PGF_{2\alpha}$ (Figure 2b)-precontracted rings with intact endothelium. Percent relaxation from baseline was not different between adrenomedullin and CGRP at any concentration (Figure 2). Neither removal of the endothelium nor L-NAME (100 μM) altered relaxant responses to adrenomedullin and CGRP in rings precontracted with histamine (10 μM) (Table 2).

$CGRP_{8-37}$ at 1 μM or 10 μM had no effect on histamine-induced tone. However, $CGRP_{8-37}$ significantly attenuated the responses to CGRP (3 nM to 30 nM); the inhibitory effects of $CGRP_{8-37}$ on CGRP-induced vasorelaxant responses were greater at a 10 μM concentration than at 1 μM (Figure 3b). In contrast, $CGRP_{8-37}$ (1 μM to 10 μM) had no effect on adrenomedullin (from 3 nM to 30 nM)-induced relaxations of vessel rings (Figure 3a).

Table 1 Concentration-response effects of adrenomedullin and calcitonin gene-related peptide on non-adrenergic non-cholinergic (NANC) and substance P-induced contractions in bronchial strips

	% inhibition			
	Adrenomedullin		Calcitonin gene-related peptide	
Concentration	NANC (10 Hz)	Substance P (1 μM)	NANC (10 Hz)	Substance P (1 μM)
1 nM	0.0±0.3	0.0±0.2	0.0±0.3	0.0±0.4
3 nM	0.0±0.2	0.0±0.6	0.0±0.5	1.0±0.8
10 nM	0.0±0.5	0.0±0.3	1.7±1.0	3.0±1.7
30 nM	0.0±0.3	0.0±0.3	6.4±2.2	9.4±2.7
100 nM	0.0±0.3	0.0±0.5	12.3±3.4*	16.1±3.9*
300 nM	0.0±0.5	0.0±0.3	20.8±3.7*	22.4±4.0*
1 μM	0.0±0.5	0.0±0.5	24.5±3.7**	28.2±5.0**

Data are shown as mean±s.e.mean of values from 7 guinea-pigs. *$P<0.05$ and **$P<0.01$ vs. control.

Discussion

Bronchomotor effect

The effects of CGRP on tracheobronchial smooth muscle are controversial. CGRP is reported to contract smooth muscle in guinea-pig trachea (Tschirhart *et al.*, 1990) and in the human bronchus (Palmer *et al.*, 1987) *in vitro*. On the other hand, CGRP is shown to have no effect on the airway smooth muscle in the guinea-pig (Bhogal *et al.*, 1994) and pig trachea (Kannan & Johnson, 1991) *in vitro* and in the sheep *in vivo* (Parsons *et al.*, 1992), and to cause inhibition of the carbachol- and 5-hydroxytryptamine-induced contraction of rat isolated airways (Cadieux *et al.*, 1990). Furthermore, CGRP is reported to cause relaxations in guinea-pig trachea precontracted with KCl and $PGF_{2\alpha}$ (Bhogal *et al.*, 1994) and in pig trachea (Kannan & Johnson, 1992) and mouse bronchus (Manzini, 1992) precontracted with carbachol. Our data show that neither CGRP nor adrenomedullin had any contractile activity on the base-

Table 2 Effects of endothelium removal and N^G-nitro-L-arginine methyl ester (L-NAME) on the relaxant response of histamine-precontracted vessel rings to adrenomedullin and calcitonin gene-related peptide

	% relaxation of histamine tone					
	Adrenomedullin			Calcitonin gene-related peptide		
Concentration	Endothelium (+)	Endothelium (−)	L-NAME (100 μM)	Endothelium (+)	Endothelium (−)	L-NAME (100 μM)
1 nM	0±0	0±0	0±0	0±0	2±1	2±1
10 nM	28±2	27±3	30±3	32±3	30±3	29±3
100 nM	51±4	56±5	49±4	52±5	54±4	50±4
1 μM	51±4	55±5	51±5	52±5	55±5	49±4

Data are shown as mean±s.e.mean of values from 7 guinea-pigs.

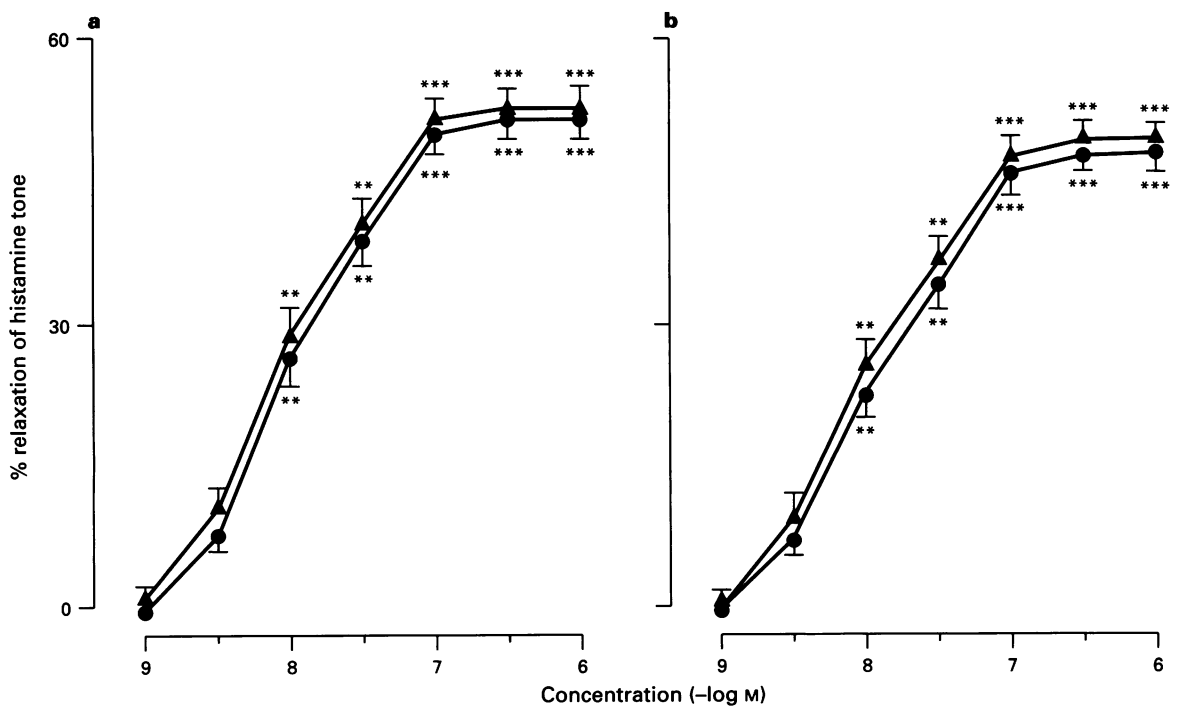

Figure 2 Relaxant effects of adrenomedullin (●) and calcitonin gene-related peptide (▲) on histamine (a) and prostaglandin $F_{2\alpha}$ (b)-precontracted vessel rings with intact endothelium. Data are shown as mean±s.e.mean of values from 7 guinea-pigs. Significant differences from corresponding control values are indicated by **$P<0.01$ and ***$P<0.001$.

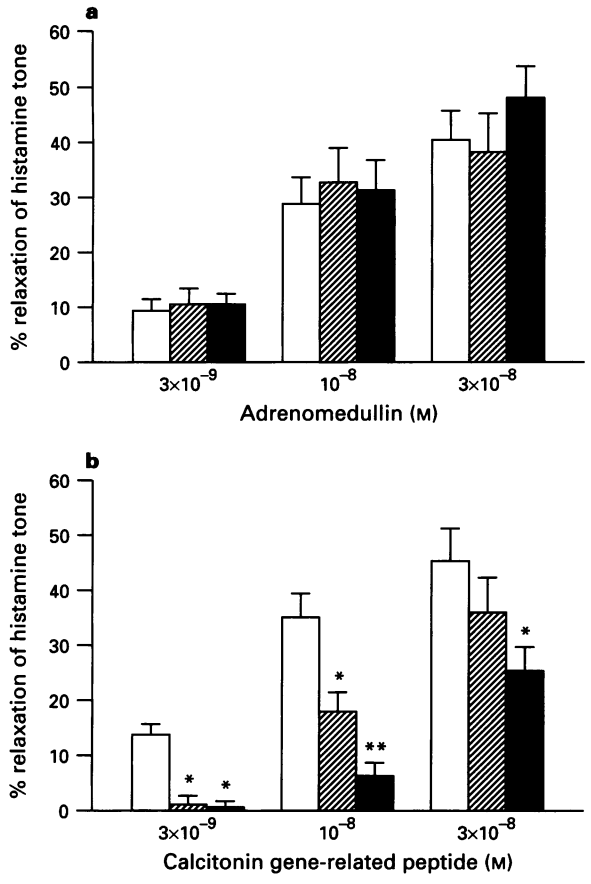

Figure 3 The effects of calcitonin gene-related peptide$_{8-37}$ ($CGRP_{8-37}$) on adrenomedullin (a) and CGRP (b)-induced relaxations of vessel rings with intact endothelium. Open columns represent controls, and hatched and closed columns represent responses in the presence of 1 μM or 10 μM concentration of $CGRP_{8-37}$, respectively. Data are shown as mean$\pm$s.e.mean values from 7 guinea-pigs. Significant differences from corresponding control values are indicated by $^*P<0.05$ and $^{**}P<0.01$.

line tone and support some previous studies (Kannan & Johnson, 1991; Bhogal *et al.*, 1994). We also show that CGRP potentiated the EFS-induced cholinergic contraction. In order to establish whether CGRP elicits its potentiating effects prejunctionally on neuronal terminals or postjunctionally on the acetylcholine receptors of the airway smooth muscle, we compared the effects of CGRP on the contractile response to EFS with the effects that they had on the contractile response to acetylcholine. CGRP potentiated contractions induced by EFS without a significant effect on contractions induced by acetylcholine. These results suggest that CGRP facilitates the release of acetylcholine from cholinergic nerves. In contrast, cholinergic contraction may not be influenced by adrenomedullin, since it had no effect on either EFS-induced cholinergic or acetylcholine-induced contractions.

CGRP reduced the excitatory NANC response evoked by EFS in guinea-pig bronchi. This excitatory response resulting from nerve stimulation in the presence of atropine is noncholinergic in nature, and probably due to the release of neuropeptides such as substance P and neurokinin A from sensory nerve endings (Lundberg *et al.*, 1983). Since CGRP reduced both EFS-induced NANC and substance P-induced contractions with similar magnitude, the inhibitory action of CGRP would occur at the postjunctional level. Our result is in accord with a previous study (Gatto *et al.*, 1989) demonstrating that CGRP blocked substance P-induced increases in pulmonary resistance in guinea-pig *in vivo*. In contrast, adrenomedullin did not alter the contractions induced by stimulation of the excitatory NANC nerves and substance P, suggesting that the peptidergic sensory nerves are not influenced by adrenomedullin.

The guinea-pig trachea receives an inhibitory NANC innervation and there is some evidence suggesting that vasoactive intestinal polypeptide (VIP) may be the transmitter (Matsuzaki *et al.*, 1980). However, when responses to VIP were abolished by treatment with VIP antiserum or peptidase, responses to stimulation of the inhibitory NANC nerves were reduced, but not abolished, indicating that a non-VIP component may be involved in the NANC stimulation-induced relaxations (Ellis & Farmer, 1989). Li & Rand (1991) have shown that a part of the non-VIP component is reduced by the nitric oxide synthesis inhibitor N^G-monomethyl L-arginine, suggesting that nitric oxide as well as VIP mediates NANC-induced relaxations of guinea-pig tracheal smooth muscle. In the present study, neither CGRP nor adrenomedullin had major effects on NANC relaxation, suggesting that these agents have no effect on relaxation induced by neurally derived VIP or nitric oxide.

Vasorelaxant effect

Adrenomedullin is reported to relax the cat isolated pulmonary arterial smooth muscle precontracted with U46619 (Gumusel *et al.*, 1995) and have a vasodilator activity on the pulmonary artery in cats and rats *in vivo* (Nossaman *et al.*, 1995). Likewise, CGRP is a potent vasodilator of the isolated pulmonary artery in man (McCormack *et al.*, 1989) and guinea-pigs (Maggi *et al.*, 1990). The vasodilator activity of the pulmonary artery of adrenomedullin was equipotent in cats and less potent in rats compared to that of CGRP (Nossaman *et al.*, 1995). The present study shows that both adrenomedullin and CGRP have a vasodilator activity on the pulmonary artery with a similar potency in guinea-pigs *in vitro*.

There has not been agreement in the literature regarding the endothelial dependence of the vasodilator action of CGRP. Many vasodilators such as acetylcholine, bradykinin, and substance P are shown to act by releasing an endothelium-derived relaxing factor (Furchgott & Zawadzki, 1980). Several investigators have demonstrated that CGRP is an endothelium-dependent vasodilator in the rat aorta (Brain *et al.*, 1985; Grace *et al.*, 1987) and in human systemic arteries (Thom *et al.*, 1987). Other investigators have reported that an intact endothelium is not required for CGRP vasodilatation in the cat cerebral artery (Edvinsson *et al.*, 1985), bovine coronary artery (Greenberg *et al.*, 1987), the pig coronary artery (Franco-Cereceda *et al.*, 1987), and the guinea-pig pulmonary artery (Martling *et al.*, 1988; Maggi *et al.*, 1990; Liu *et al.*, 1992; Butler *et al.*, 1993). The endothelium-derived relaxing factor has now been identified as nitric oxide (Palmer *et al.*, 1987; Moncada *et al.*, 1991). Therefore, our *in vitro* results, showing the nonendothelial dependence of vasodilatation of CGRP and adrenomedullin in pulmonary arteries of guinea-pigs, are in agreement with the findings that the nitric oxide synthesis inhibitor L-NAME failed to alter the vasorelaxant effects of adrenomedullin and CGRP.

Vasodilator responses to CGRP are readily antagonized by a CGRP receptor antagonist, $CGRP_{8-37}$ (Chiba *et al.*, 1989). Thus, $CGRP_{8-37}$ inhibited relaxation responses to CGRP in the rat isolated perfused kidney (Castellucci *et al.*, 1993; Chin *et al.*, 1994), perfused rat mesenteric arterial bed (Han *et al.*, 1990) and porcine coronary artery (Franco-Cereceda, 1991). $CGRP_{8-37}$ also inhibited vasodilatation elicited by CGRP *in vivo* in the rabbit (Hughes & Brain, 1991) and rat skin beds (Escott & Brain, 1993) and in cat cerebral arterioles (Wei *et al.*, 1992). In conscious rats, $CGRP_{8-37}$ inhibited vasodilator responses to CGRP in the renal and hindquarter beds and vasoconstrictor response to CGRP in the mesenteric bed (Gardiner *et al.*, 1990; 1995). The ability of $CGRP_{8-37}$ to block vasorelaxant actions of CGRP but inability to block vasodilator effects of adrenomedullin in the present study suggest that the pulmonary arterial vasodilator effect of adrenome-

dullin is not mediated via stimulation of $CGRP_1$ receptors. Recent studies (Elhawary *et al.*, 1995; Gardiner *et al.*, 1995) show that $CGRP_{8-37}$, at a dose which blocks vascular responses to CGRP, did not block the vasodilator effects of adrenomedullin in the renal beds in rats *in vivo*. These data are consistent with the present findings.

In conclusion, our study suggests that both adrenomedullin and CGRP are potent vasodilators of the pulmonary artery. Since $CGRP_{8-37}$ did not inhibit the vasodilator actions of adrenomedullin, it is suggested that these actions are not mediated via activation of $CGRP_1$ receptors. Although CGRP has a small effect on cholinergic and excitatory NANC contractions, adrenomedullin does not have any significant effects on bronchomotor tone. Therefore, the action of adrenomedullin seems to be selective on vascular smooth muscle in the lung and it may be useful for the treatment of patients with pulmonary hypertension.

We thank Professor H. Matsuo for a gift of adrenomedullin and Mr G. Crittenden for correcting the manuscript.

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(Received June 17, 1996
Revised July 26, 1996
Accepted September 9, 1996)