Carbamylcholine- and 5-hydroxytryptamine-induced contraction in rat isolated airways: inhibition by calcitonin gene-related peptide

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1 The effects of rat and human α -calcitonin gene-related peptide (α -CGRP) were investigated in isolated smooth muscle preparations obtained from three levels of the rat respiratory tract.

2 Neither peptide (10^{-10} – 10^{-6} M) had any effect on resting tension or on carbamylcholine (10^{-6} M)-induced tone of trachea or main bronchus. In contrast, CGRP sometimes reduced spontaneous or carbamylcholine-induced tone of lung parenchymal strips.

3 CGRP produced a significant rightward shift of the log concentration-response curves to carbamylcholine and 5-hydroxytryptamine (5-HT) in the main bronchus. A rightward shift was also seen in trachea and parenchymal strips but this did not achieve the level of significance. The maximal response to 5-HT was reduced in the main bronchus and lung parenchyma whereas the maximal contraction to carbamylcholine was decreased in parenchymal strip only.

4 In all three airway preparations, CGRP caused concentration-dependent inhibition of responses elicited by challenges with 10^{-7} M carbamylcholine or 5×10^{-7} M 5-HT. The inhibitory effect of the peptide was inversely related to the size of the airways: the smaller the calibre, the greater the inhibition.

5 The inhibitory action of CGRP was not modified by pretreatment with tetrodotoxin (10^{-6} M), propranolol (10^{-6} M) or indomethacin (10^{-6} M).

6 The results strongly suggest that (a) CGRP has a nonspecific inhibitory action on airway smooth muscle cells, (b) CGRP may act as a potent inhibitor of responses elicited by bronchoconstrictor substances and (c) its inhibitory activity may be most powerfully expressed in peripheral regions of the respiratory tract.

Introduction

Calcitonin gene-related peptide (CGRP) is a mammalian peptide containing 37 amino acid residues with a disulphide bridge between Cys₂ and Cys₇. It is generated by tissue-specific RNA processing of the primary transcript of the calcitonin gene (Amara *et al.*, 1982) and is expressed in thyroid parafollicular cells as well as in nuclei and nerve fibres of the central and peripheral nervous systems (Rosenfeld *et al.*, 1983). In both rat and human, this peptide is known to occur in two forms, designated α - and β -CGRP, which differ in their primary sequence by one and three amino acids respectively (Breimer *et al.*, 1988).

Using radioimmunoassay and immunocytochemical techniques, we have previously demonstrated that high concentrations of α -CGRP are found in the rat lung where the peptide exhibits a unique and widespread distribution, being the first peptide to be localized both in peripheral nerves and endocrine cells (Cadieux *et al.*, 1986a). CGRP-immunoreactive nerve fibres were found to be mostly sensory in nature. Their cell bodies lie in the nodose, jugular and dorsal root ganglia and axodendritic processes are carried in the vagus and the sympathetic nerves to form peripheral branches in the lung (Springall *et al.*, 1987). The physiological significance of CGRP in rat lung remains unclear, but the close association of CGRP-positive nerves with smooth muscle of the tracheo-bronchial tree suggests that this peptide may be involved in regulating bronchomotor tone. Recently, studies have established the presence of specific binding sites for CGRP in areas in which immunoreactivity was localized (Carstairs, 1987). These findings support the hypothesis that CGRP may participate in bronchomotor regulation by acting as a neurotransmitter or neuromodulator substance.

The present experiments were carried out to determine whether CGRP had any direct effect on the smooth muscle of

rat isolated airways. The ability of CGRP to inhibit contraction induced by carbamylcholine and 5-HT was also investigated. A preliminary account of some of this work was presented at the APS/ASPET Joint Meeting, 1988. (Cadieux *et al.*, 1988).

Methods

Tissue preparation

Male Sprague Dawley rats weighing 200–225 g were killed by exsanguination following intraperitoneal injection of pentobarbitone sodium (50 mg kg⁻¹). The trachea and the lungs were quickly removed and placed in cold Krebs solution. The trachea was separated at its junction with the main bronchi, and tracheal chains were prepared from the intrathoracic part of the trachea according to the method of Castillo & De Beer (1947). Both right and left main bronchi were dissected out and cut helically as described by Sirois *et al.* (1987). Finally, parenchymal strips (approximately 15 mm × 3 mm × 3 mm) were cut from the periphery of either right or left lower lobes of the lungs (Lulich *et al.*, 1976). Each preparation was mounted in a 5 ml jacketed organ bath containing Krebs solution gassed with 95% O₂, 5% CO₂ and maintained at 37°C. Contractions were measured isometrically with a Grass FT03 force displacement transducer and recorded on a Grass polygraph (Model 7D) as tension changes (g). All tissues were subjected to an initial loading tension of 1 g and allowed to equilibrate for 60 min (with changes of bath medium every 15 min) before CGRP and other agents were tested.

Functional studies

The effect of CGRP on the three different tissue preparations, initially was examined by testing cumulatively-applied CGRP (10^{-10} – 10^{-6} M) against the resting tension and against tension induced by carbamylcholine (10^{-6} M).

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To test for inhibitory activity of CGRP in isolated airways, cumulative concentration-response (C/R) curves to carbamylcholine (10^{-8} – 10^{-3} M) or 5-HT (10^{-8} – 10^{-4} M) were constructed in the presence of CGRP (10^{-7} M), added to the organ bath 2 min before constructing the C/R curve. Control experiments were performed in parallel on isolated airways where only the peptide vehicle (Krebs solution) was added.

Finally, the inhibitory action of CGRP was further characterized by studying its effect on contractions induced by single concentration of the two bronchoconstrictor agents. Contractions were elicited by carbamylcholine (10^{-7} M) or 5-HT (5×10^{-7} M) at 15 min intervals with a drug contact time just sufficient to record the peak response. CGRP (10^{-10} – 10^{-6} M) was then added to the organ bath and 2 min later a further spasmogen challenge occurred. The contraction produced in the presence of the peptide was then compared with the mean of two control responses to carbamylcholine or 5-HT observed prior to CGRP administration. A further concentration of CGRP was tested only when responses to the spasmogen showed no further recovery. Concentrations of the peptide were tested in a random order in each experiment. In this latter series of experiments, the inhibitory effect of CGRP was also examined in the presence of tetrodotoxin (10^{-6} M), propranolol (10^{-6} M) or indomethacin (10^{-6} M).

Calculations

On all isolated airway preparations, C/R curves were plotted as molar concentration of drug versus contractile response expressed as g of tension. EC_{50} values were calculated by linear regression analysis of all points between 20 and 80% of maximal response and expressed as pD_2 . pD_2 values were calculated from each individual C/R curve and the mean $\pm$ s.e.mean calculated from these data. The inhibitory effect of CGRP was assessed by comparing pD_2 values in the absence and the presence of CGRP. Results are presented as means $\pm$ s.e.mean. Throughout, mean values were compared by use of Student's *t* test for paired or unpaired data as appropriate. A probability level of $P < 0.05$ was considered statistically significant.

Drugs and solutions

The Krebs solution had the following composition (mM): NaCl 118.1, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11.1. The following drugs were used: synthetic rat and human α -CGRP (IAF Biochem), carbamylcholine chloride, histamine dihydrochloride, indomethacin, propranolol hydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), atropine sulphate and tetrodotoxin (TTX) (each Sigma). Methysergide bimaleate was a generous gift from Sandoz Ltd (Basel, Switzerland). All drugs were freshly diluted in the stock Krebs solution with the exception of the peptides. The latter agents, in lyophilized form, were dissolved in distilled water at an initial concentration of 1 mg ml^{-1} and kept frozen at -20°C . The required working solutions were made by dilution in the stock Krebs solution immediately before the experiments. All concentrations referred to are final bath concentrations. The glassware (including the organ baths) were siliconised (Prosil-28) in order to minimize non specific adsorption.

Results

Effect of CGRP on spontaneous and carbamylcholine-induced tone

Figure 1(a,b) shows that the resting tension of rat main bronchus was not affected by either rat or human α -CGRP (10^{-10} – 10^{-6} M). Neither peptide suppressed contraction induced by 10^{-6} M carbamylcholine in this preparation. Similar results were obtained with rat trachea. However, in

Figure 1 Original recordings showing the effects of increasing concentrations of rat and human α calcitonin gene-related peptide (α -CGRP) on the resting tension (a,c,d) and on carbamylcholine-induced tone (b,e) of rat isolated airways. Upper panel: main bronchus. Lower panel: parenchymal strips. Cumulative additions of CGRP (Δ) at concentrations from 10^{-10} to 10^{-6} M did not exert any contractile or relaxant effect in main bronchus. Note however, that some parenchymal strips relax when challenged with CGRP (d,e) while others fail to respond (c). Ordinate scale: changes of tension in g. Abscissa scale: time in min. W: washouts. Arrows: carbamylcholine (10^{-6} M). r-CGRP: rat α -CGRP. h-CGRP: human α -CGRP.

about 40% of the lung parenchymal strips, CGRP caused a significant reduction of spontaneous tone (Figure 1d). When these same preparations were pre-contracted with carbamylcholine 10^{-6} M, CGRP caused concentration-dependent relaxation (Figure 1e). CGRP-induced relaxation was not reduced by tetrodotoxin (10^{-6} M) propranolol (10^{-6} M) or indomethacin (10^{-6} M).

Effect of CGRP on concentration-response curves of carbamylcholine and 5-HT

The addition of 5-HT (10^{-8} – 10^{-4} M) or carbamylcholine (10^{-8} – 10^{-3} M) caused, in all three preparations, rapidly-developing and concentration-dependent contraction. 5-HT and carbamylcholine were strongly antagonized by methysergide (10^{-6} M) or atropine (5×10^{-6} M) respectively. Both carbamylcholine and 5-HT were more potent on the trachea and main bronchus than on lung parenchymal strips (Table 1). Histamine had no effect on the resting tension of the same preparations.

Preincubation of the airways with 10^{-7} M of rat α -CGRP resulted in a significant shift to the right of the carbamylcholine C/R curve in the main bronchus but not in trachea and parenchymal strips (Figure 2, Table 2). In the trachea and main bronchus the maximal responses to carbamylcholine were not affected by the presence of CGRP whereas in lung parenchymal strips the presence of the peptide caused a 31.4% reduction in the maximal contraction. Although the rightward shift for carbamylcholine was not sig-

Table 1 pD_2 values and developed maximal contraction (m.c.) for carbamylcholine and 5-hydroxytryptamine (5-HT) in tissues isolated from rat lung

		Carbamylcholine	5-HT
Trachea	pD_2	6.71 ± 0.03	6.15 ± 0.03
	m.c. (g)	0.36 ± 0.03 (6)	0.175 ± 0.015 (14)
Main bronchus	pD_2	6.49 ± 0.03	5.89 ± 0.02
	m.c. (g)	1.23 ± 0.1 (6)	0.37 ± 0.03 (17)
Parenchyma	pD_2	5.34 ± 0.09	5.29 ± 0.06
	m.c. (g)	0.102 ± 0.006 (10)	0.133 ± 0.009 (7)

The pD_2 values are expressed as mean $\pm$ s.e.mean. Figures in parentheses indicate the number of tissues.

Figure 2 Cumulative concentration-response curves for carbamylcholine in the absence (○) and in the presence (●) of calcitonin gene-related peptide (CGRP, 10^{-7} M): (a) trachea, (b) main bronchus and (c) parenchymal strips. The peptide was added to the organ bath 2 min prior to the administration of the cholinergic agonist. Values are expressed as g tension. Each point and bar represents the mean and s.e. mean of data from 4 to 12 tissues. Asterisks: significantly different from controls.

nificant in parenchymal strips, responses of both low and high concentrations of the cholinergic agonist were diminished in the presence of CGRP. This differs from the main bronchus where only contractile responses in the lower third of the C/R curve were decreased by pretreatment with the peptide.

5-HT-induced contractions were similarly affected by CGRP (Figure 3, Table 2) except that CGRP caused a significant reduction (30.8%) in the maximal response to 5-HT in the main bronchus. In parenchyma, the decrease in the

Figure 3 Cumulative concentration-response curves for 5-hydroxytryptamine (5-HT) in the absence (○) and in the presence (●) of calcitonin gene-related peptide (CGRP, 10^{-7} M): (a) trachea, (b) main bronchus and (c) parenchymal strips. The peptide was added to the organ bath 2 min prior to the administration of 5-HT. Values are expressed as g tension. Each point and bar represents the mean and s.e. mean of data from 8 to 16 tissues. Asterisks: significantly different from controls.

maximal contraction following the administration of CGRP, averaged 42.1%.

Effect of various concentrations of CGRP on responses to single concentrations of carbamylcholine and 5-HT

The results presented above suggest that the modulatory effect of CGRP on the contractions induced by the two spasmogens may differ with respect to target tissue (distal versus proximal airways). An increase in the carbamylcholine concentration overcame the inhibitory effect of CGRP in the main bronchus

Table 2 pD_2 values for carbamylcholine 5-hydroxytryptamine (5-HT) and rat airways in the absence and presence of calcitonin gene-related peptide (CGRP) 10^{-7} M

Spasmogen	Target tissue	Control pD_2	CGRP pD_2	Significance level
Carbamylcholine	Trachea	6.71 ± 0.03 (6)	6.68 ± 0.06 (4)	NS
	Main bronchus	6.49 ± 0.03 (6)	6.38 ± 0.03 (4)	$P < 0.05$
	Parenchyma	5.34 ± 0.09 (10)	5.23 ± 0.07 (12)	NS
5-HT	Trachea	6.15 ± 0.03 (12)	6.02 ± 0.06 (11)	NS
	Main bronchus	5.89 ± 0.02 (16)	5.72 ± 0.08 (8)	$P < 0.05$
	Parenchyma	5.29 ± 0.06 (7)	5.39 ± 0.08 (8)	NS

Each value is the mean $\pm$ s.e.mean. Figures in parentheses indicate the number of tissues. NS: not significant.

but not in lung parenchymal strips. Similarly an increase in the concentration of 5-HT overcame CGRP-induced inhibition in the trachea but not in the main bronchus or parenchymal strip. This issue was further investigated by comparing the inhibitory effect of increasing concentrations of CGRP on carbamylcholine- and 5-HT-induced contractions in all three airway preparations.

The spasmogen challenges (10^{-7} M for carbamylcholine; 5×10^{-7} M for 5-HT), induced contractions varying between 5 and 20% of maximal responses depending on the airway preparation used. As illustrated in Figure 4, preincubation of rat bronchus with rat α -CGRP (10^{-10} M) caused a slight reduction of carbamylcholine-induced contraction but did not alter either the amplitude or the shape of the contractile response to added 5-HT. In contrast, pretreatment with higher concentrations of the peptide significantly decreased the contractions induced by the two bronchoconstrictor agents (Figures 4 and 5). The effect of CGRP did not show tachyphylaxis and the inhibitory action was rapidly reversible at lower doses of the peptide. In contrast, the inhibitory effect of high doses of CGRP ($>10^{-8}$ M) tended to persist for 30 to 45 min despite repeated washings. Generally, in each preparation from one animal, the same dose of CGRP added repeatedly during the experiment, produced similar inhibition of the contraction.

The inhibitory effects of increasing concentrations of CGRP on responses to 10^{-7} M carbamylcholine and 5×10^{-7} M 5-HT are summarized in Figures 5a and 5b. As shown in Figure 5a, rat α -CGRP added 2 min before carbamylcholine inhibited contractions of the airways in a concentration-dependent manner. The inhibitory effect of the peptide was more marked in the lower segments of the tracheobronchial tree than in the trachea and, at a concentration of 10^{-6} M, reached an inhibitory effect of $63.3 \pm 5.9\%$ ($P < 0.05$) in the

Table 3 Effect of tetrodotoxin, indomethacin and propranolol on the inhibitory action of calcitonin gene-related peptide (CGRP) on carbamylcholine- and 5-hydroxytryptamine (5-HT)-induced contractions in rat isolated parenchymal strips

	% inhibition of spasm induced by CGRP (10^{-7} M)	
	Carbachol (10^{-7} M)	5-HT (5×10^{-7} M)
Control	51.2 ± 3.1 (5)	39.9 ± 6.2 (3)
Tetrodotoxin (10^{-6} M)	49.5 ± 2.6 (3)	37.4 ± 2.5 (3)
Indomethacin (10^{-6} M)	51.7 ± 8.4 (5)	45.1 ± 7.7 (4)
Propranolol (10^{-6} M)	48.6 ± 6.7 (4)	40.7 ± 2.1 (3)

Each value is the mean $\pm$ s.e.mean. Figures in parentheses indicate the number of tissues.

parenchymal strips, $32.5 \pm 3.2\%$ ($P < 0.05$) in the main bronchus and $7.3 \pm 3.3\%$ ($P > 0.05$) in the trachea. Similar results were obtained with 5-HT as the spasmogen. Pretreatment with 10^{-6} M CGRP, 2 min before the administration of 5×10^{-7} M 5-HT decreased the agonist-induced contractions by $42.6 \pm 3.8\%$ in lung parenchyma ($P < 0.05$), $31.3 \pm 3.8\%$ in main bronchus ($P < 0.05$) and $13.4 \pm 0.65\%$ in trachea ($P > 0.05$) of the controls, respectively (Figure 5b).

Human α -CGRP was equipotent with rat α -CGRP and exhibited similar inhibitory activity on contractions induced by carbamylcholine and 5-HT in all segments of the respiratory tract (Figures 5a and b). Finally, the inhibitory effect of rat α -CGRP on airways contractility was unchanged after

Figure 4 Recordings of the inhibitory effect of rat α -calcitonin gene-related peptide (α -CGRP) on contractions evoked by 10^{-7} M carbamylcholine (a) and by 5×10^{-7} M 5-hydroxytryptamine (b) in rat isolated main bronchus. Contractions were elicited by the two agonists at 15 min intervals in the absence or in the presence of various concentrations of CGRP. Note that the inhibitory action of CGRP tended to persist longer when high concentration (10^{-7} or 10^{-6} M) was added to the organ bath. In each panel, the two recordings were obtained from the same tissue. Ordinate scale: changes of tension in g. Abscissa scale: time in min. W: washouts.

Figure 5 Log concentration-response curves for calcitonin gene-related peptide (CGRP) in inhibiting contractile responses to (a) carbamylcholine (10^{-7} M) and (b) 5-hydroxytryptamine (5-HT, 5×10^{-7} M) in rat isolated airways. CGRP was added in a manner similar to that described in the legend to Figure 4. Ordinate scale: degree of inhibition produced by CGRP expressed as percentage of contraction developed by the agonist alone. Abscissa scale: concentration of CGRP. Each point and bar represents the mean and s.e. mean of data from 3 to 10 tissues. *Contractile responses were elicited by 10^{-6} M carbamylcholine. Solid line and symbols: rat α -CGRP, broken line with open symbols: human α -CGRP.

20 min pretreatment with tetrodotoxin (10^{-6} M), propranolol (10^{-6} M) or indomethacin (10^{-6} M) (Table 3).

Discussion

The results obtained in this study revealed that rat and human α CGRP exert no direct contractile effect in rat isolated trachea and main bronchus. However, CGRP sometimes decreased spontaneous or carbamylcholine-induced tone of lung parenchymal strips. In agreement with Lulich & Paterson (1980) and Vornanen & Tirri (1981), all of these three classical airway smooth muscle preparations were insensitive to histamine but contracted in a concentration-dependent manner to carbamylcholine and 5-HT, both agonists being more potent in the trachea and main bronchus than in lung parenchymal strips. The apparent lack of responsiveness of

the rat proximal airways to CGRP also corroborates previous reports that CGRP does not affect the resting tension of rat trachea *in vitro* (Hamel & Ford-Hutchinson, 1988) nor guinea-pig tracheal and bronchial smooth muscle *in vitro* (Martling *et al.*, 1988) and *in vivo* (Lundberg *et al.*, 1985).

In contrast to the findings of Hamel & Ford-Hutchinson (1988), our study showed that about 40% of the lung parenchymal strip preparations relaxed in response to CGRP. A likely explanation for this action of the peptide could be that different lung strips from the same or from different rats contained different proportions of vascular and airways smooth muscle (Lulich *et al.*, 1976; Goldie *et al.*, 1982; Bertram *et al.*, 1983). Furthermore, it has been reported that vascular smooth muscle may contribute significantly in responses of this preparation to agonists which stimulate airways smooth muscle (Mirbahar & Eyre, 1980; Evans & Adler, 1981). Since CGRP is a potent vasodilator agent in several species including man (Brain *et al.*, 1985; Gennari & Fischer, 1985) and causes relaxation of isolated pulmonary arteries (McCormack *et al.*, 1988; Martling *et al.*, 1988) and since CGRP does not affect the resting tension of isolated trachea and hilus bronchi of rodents (Lundberg *et al.*, 1985; Martling *et al.*, 1988; Hamel & Ford-Hutchinson, 1988; present study) we suggest that the relaxation induced by CGRP in some parenchymal preparations was due to the presence of a relatively higher proportion of vascular than airways smooth muscle cells. This relaxation was not blocked by tetrodotoxin, propranolol or indomethacin which is consistent with the finding that CGRP relaxes blood vessels by acting via its own receptors located on the vascular smooth muscle cells (Brain *et al.*, 1985). These parenchymal strips were thus considered not to reflect the activity of the smooth muscle of the fine peripheral airways and all parenchymal strips displaying a relaxation in response to 10^{-7} M CGRP were eliminated and not used in further experiments.

The present study provides strong evidence that CGRP is not totally devoid of biological effect on rat airways smooth muscle. Concentration-response curves to carbamylcholine and 5-HT were displaced to the right by CGRP in the main bronchus. Corresponding curves in the trachea and parenchymal strip were altered in shape. In addition, the CGRP-induced inhibition of contractile responses to the two spasmogens was inversely related to the size of the airways; the smaller the calibre the greater the inhibition. CGRP-induced reduction of responses to carbamylcholine and 5-HT standards (10^{-7} and 5×10^{-7} M respectively) was consistent with its downward displacement effect on the C/R curves of the agonists. Its inhibitory action was concentration-dependent and was most powerfully expressed on parenchymal strips and least powerfully expressed on trachea. These results are consistent with a neurotransmitter role for CGRP in regulating airway smooth muscle tone.

The finding that the magnitude of the inhibitory effect of CGRP on carbamylcholine- or 5-HT-induced contraction was greatest in parenchymal strips and smallest in trachea corresponds with the reported pattern of distribution of CGRP binding sites in rodent lungs. CGRP receptor sites are abundant in intrapulmonary bronchi of all sizes including the alveolar ducts but are sparsely distributed in smooth muscle of large airways (Carstairs 1987; Mak & Barnes, 1988). CGRP may therefore be of functional importance mainly in the periphery of the respiratory tract; hence providing confirmation of the heterogeneity of smooth muscle responsiveness through the lung (Burns & Doe, 1978; Lulich & Paterson, 1980; Vornanen & Tirri, 1981). The inhibitory action of CGRP was expressed against both carbamylcholine- and 5-HT-induced contraction and was not mediated by the release of endogenous prostaglandins since indomethacin, an inhibitor of prostaglandin synthesis, had no effect. Moreover, the activity of CGRP was not modified by tetrodotoxin (10^{-6} M) or propranolol (10^{-6} M) which indicates that the action of CGRP is not related to the discharge of neuronal action potentials or to β -adrenoceptor activation consequent to noradrenaline

release. These results suggest instead that CGRP exerts a direct, non-specific inhibitory action on the smooth muscle cells. Furthermore, the low threshold dose required to induce an effect as well as the long duration of action of CGRP (especially noted by the slow recovery of the tissue following the administration of high doses of the peptide) suggest a high affinity of CGRP for its receptor. This would be in keeping with the demonstration of receptors with high affinity for CGRP in airway smooth muscle of mammalian lung (Mak & Barnes, 1988).

There is some suggestion that rat and human α -CGRP may differ slightly in biological activity (Marshall *et al.*, 1986) but the structure-activity relationships have not yet been examined in detail. According to our observations both peptides are equipotent in inducing inhibition of agonist-induced contractions in rat isolated airways. This is consistent with previous findings in the cardiovascular system where both rat and human α -CGRP behave similarly (Brain *et al.*, 1986; Tippins *et al.*, 1986). This suggests that the substitutions in position 1, 3, 25 and 35 in the amino acid sequence between rat and human α -CGRP (Rosenfeld *et al.*, 1983; Breimer *et al.*, 1988) have little or no effect on the biological activity of the peptide at least in some organs and/or tissues.

Evidence is accumulating that CGRP may be an important neurotransmitter in the respiratory system. In animals and man CGRP is present in sensory nerves which are associated either with vascular and non vascular smooth muscle of the lung (Rosenfeld *et al.*, 1983; Lundberg *et al.*, 1985; Uddman *et al.*, 1985; Cadieux *et al.*, 1986a; Martling *et al.*, 1988). CGRP is released from the lung upon stimulation with capsaicin (Martling *et al.*, 1988) and following cigarette smoke exposure (Cadieux *et al.*, 1986b). There has been much debate about the possible role of CGRP in the respiratory system but agreement exists between authors that a common effect of the peptide is to regulate blood flow (Joos *et al.*, 1986; Lundberg

& Saria, 1987; Laitinen *et al.*, 1987; Uddman & Sundler, 1987). Hence, CGRP relaxes isolated pulmonary arteries obtained from both rodents (McCormack *et al.*, 1988) and human lungs (Martling *et al.*, 1988) and potentiates tachykinin-induced vascular permeability in dog (Laitinen *et al.*, 1987) and guinea-pig trachea (Lundberg *et al.*, 1985; Haass & Skofitsch, 1985). The involvement of CGRP in the regulation of the airway smooth muscle currently remains unclear. In human isolated airway smooth muscle there is evidence for (Palmer *et al.*, 1987) and against (Martling *et al.*, 1988) a bronchoconstrictor action of CGRP. Similarly, Lundberg *et al.*, (1985) and Martling *et al.*, (1988) showed that CGRP does not exert any contractile or relaxant effect in guinea-pig trachea and hilus bronchi, whereas Hamel & Ford-Hutchinson (1988) claimed that CGRP is one of the most potent contractile agents on guinea-pig trachea yet described. Results of the present study suggest that CGRP has an anti-bronchoconstrictor action in rat isolated airways, and is consistent with the report of Gatto *et al.* (1989) that CGRP potentially inhibits the bronchoconstrictor effects of substance P and bombesin in the guinea-pig *in vivo*.

In summary, our findings indicate that CGRP exerts an inhibitory effect against bronchoconstrictor agents in rat isolated airways. This inhibitory effect is greatest in the periphery of the tracheobronchial tree and represents a direct effect of CGRP on the membrane of the smooth muscle cells. These observations support the hypothesis that CGRP may play a role in regulating airway tone.

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