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Gastrin Releasing Peptide (GRP) Binding Sites in Human Bronchi

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Abstract—The autoradiographic binding site of gastrin releasing peptide (GRP), the 27 amino acid mammalian form of bombesin, were examined in human bronchial mucosa. ^{125}I-GRP bound specifically to submucosal glands and the epithelium. There was limited binding to vessels and bronchial smooth muscle. These observations suggest that GRP or GRP immunoreactive peptides which are present in nerve fibres and pulmonary neuroendocrine cells, may act upon glandular GRP receptors to induce mucus secretion, but that GRP would probably have little effect on vascular permeability or tracheobronchial smooth muscle tone.

Introduction

Gastrin releasing peptide (GRP) is a 27 amino acid (2859 g/mol) mammalian peptide (1). The C-terminal decapeptide, which is essential for receptor recognition and biological activity, shares sequence homology with bombesin, a 14 amino acid amphibian peptide (2). Other GRP immunoreactive peptides (GRP-irp) include GRP[18-27] and unidentified peptides in human bronchoalveolar lavage fluid (3). GRP-irp are located in nerve fibres (1, 4) and pulmonary neuroendocrine cells (1), and may act as neurotransmitters (5) and neuroregulatory hormones (1, 6). GRP and bombesin are growth factors in fetal (7), normal (8), and neoplastic (9) respiratory tissues (1). GRP-irp are produced by small cell lung carcinoma cell lines (10). Bombesin stimulate glandular secretion from both human and guinea pig nasal mucosa in vivo (11, 12). In vitro, GRP stimulates mucus secretion from cat trachea (13) and human nasal mucosa (14). Both lactoferrin, a product of submucosal gland serous cells (15), and ^{3}H-glucosamine-labelled respiratory glycoconjugates were released from human nasal mucosa (14). GRP is also a stimulant of pancreatic exocrine secretion (16) and a participant in the central and vagal control of gastric mucosal homeostasis (17).

In order to examine the potential roles of GRP in the lower respiratory tract, ^{125}I-GRP binding sites were localized in human bronchial mucosa using autoradiography.

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Methods

Human lung specimens were obtained from patients undergoing thoracotomy for carcinoma.

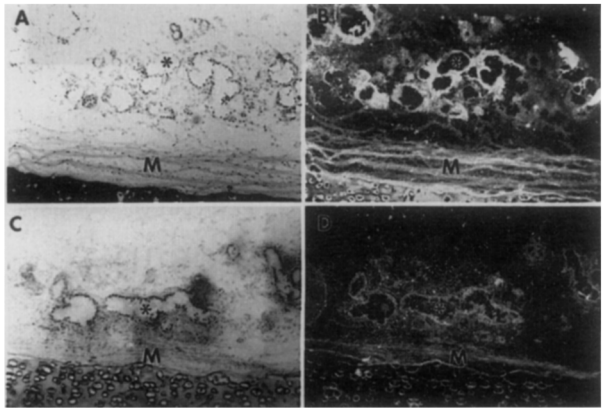

Fig. 1 ^{125}I-GRP binding sites in human bronchial mucosa. (A) Brightfield image of submucosal glands (*), tracheobronchial smooth muscle (M) and cartilage counterstained with toluidine blue. The bar represents 100 μm. (B) Darkfield image of (A) showing ^{125}I-GRP binding to submucosal glands (*). The silver grains are very dense over the glands. Limited numbers of grains are seen over the bronchial smooth muscle (M). (C) Brightfield image of an adjacent section treated with excess GRP. (D) Darkfield image of (C) demonstrating that the addition of excess GRP ablated the binding of ^{125}I-GRP to the glands (*) and smooth muscle (M).

Results

^{125}I-GRP bound specifically to the epithelium and submucosal glands (Figs 1, 2, and 3). Submucosal glands expressed ^{125}I-GRP binding sites most prominently (Figs 1 and 2). Both mucous and serous cells of submucosal glands bound ^{125}I-GRP. Secretory ducts also expressed ^{125}I-GRP binding sites. Epithelial cells diffusely bound ^{125}I-GRP, and there was no apparent preference for goblet cells or other cell populations (Fig. 3). The silver grain density was higher over glands than over epithelium, and longer time exposures were required to see grain development over the epithelium suggesting that the ^{125}I-GRP binding site density was higher in the glands. Vessel walls exhibited very limited ^{125}I-GRP binding (Fig. 3). The types of vessels and cells containing the GRP binding sites could not be determined. Bronchial smooth muscle also displayed limited ^{125}I-GRP binding (Fig. 1). The addition of excess GRP ablated the binding ^{125}I-GRP to submucosal glands, epithelium, vessels, and smooth muscle indicating the specificity of the binding sites.

Blocks of perihilar tissue were frozen in 2-methylbutane for 30 s and stored at −70 °C until required. Cryostat sections (10 μm thick) were thaw mounted onto gelatinized slides, and warmed to room temperature. The slides were washed in CMRL media with aprotinin (400 kallikrein inhibitory units per ml, Sigma Chemical Co., St. Louis, MO, USA) and 0.5% bovine serum albumin for 30 min at 25 °C (13, 14). Slides were incubated for 75 min at 4 °C with 1 nM 3-[^{125}I]-tyr^{15}-GRP (Amersham, Arlington Heights, IL, USA) in CMRL/aprotinin/BSA. Specific binding was determined by adding 1 nM ^{125}I-GRP with and without 2 μM unlabelled GRP. After incubation, slides were washed 4 times each for 30 s with CMRL/aprotinin/BSA at 4 °C and dried with cold, dry air. Slides were dipped in melted nuclear track emulsion (NTB-2, Eastman Kodak, Rochester, NY, USA) diluted 1:1 with water and stored at 4 °C in light tight boxes. At intervals emulsion coated slides were removed and developed in Kodak reagents. Slides were counterstained with toluidine blue prior to darkfield microscopy.

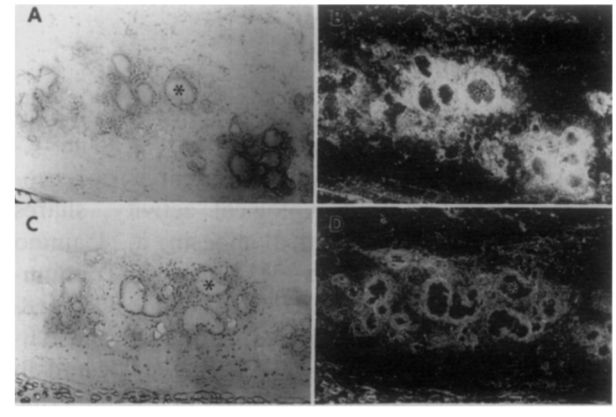

Fig. 2 ^{125}I-GRP binding sites in human bronchial mucosa. (A) Brightfield image of submucosal glands (*) counterstained with toluidine blue. The bar represents 100 μm. (B) Darkfield image of (A) showing a very high density of silver grains indicating ^{125}I-GRP binding to both the mucous and serous cells of the submucosal glands (*). The silver grains are very dense over the glands. (C) Brightfield image of an adjacent section treated with excess GRP. (D) Darkfield image of (C) demonstrating that the addition of excess GRP ablated the binding of ^{125}I-GRP to the glands (*).

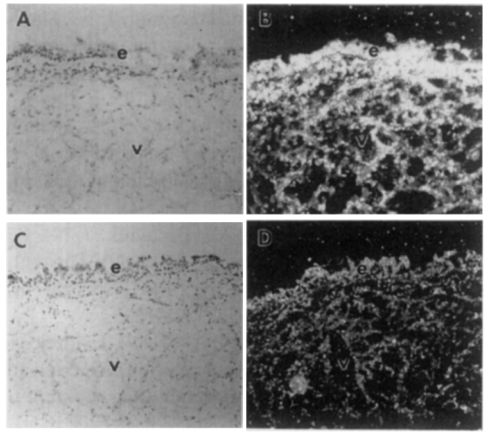

Fig. 3 ^{125}I-GRP binding sites in human tracheobronchial mucosa. (A) Brightfield image of the epithelium (e) and submucosal plexus of vessels (v). The bar represents 100 μm. (B) Darkfield autoradiographic image (A) showing silver grains which represent ^{125}I-GRP binding sites. Silver grains are seen over the epithelium (e) and vessels walls (v). (C) Brightfield image of an adjacent section treated with excess GRP. (D) Darkfield autoradiographic image of (C). The addition of 1 μM GRP ablated the specific binding of ^{125}I-GRP to the epithelium (e) and vessels (v).

Discussion

^{125}I-GRP binding sites abounded on submucosal glands and the epithelium. This finding is consistent with the known functions of GRP. GRP and bombesin are potent stimuli of serous cell, mucous cell and possibly goblet cell secretion from human, cat and guinea pig upper and lower respiratory mucosa in vitro and in vivo (11-14). Bombesin nasal provocation in humans induces both serous and mucous cell secretion without increasing vascular permeability (11). Bombesin is a vasoconstrictor in canine tracheal vessels (18), and can stimulate bronchoconstriction in the guinea pig (19).

Because of the limited supply of human tissue, it was not possible to examine the capacity of GRP to stimulate glandular secretion, vascular permeability or muscle contraction in human tracheobronchial tissue or to quantify receptor numbers. However, the distribution of ^{125}I-GRP binding sites suggests that GRP would preferentially affect glandular and epithelial cell functions.

The potential sources of GRP-irp in human pulmonary tissue are diverse. Neuroendocrine cells may be the primary source in humans (3, 20, 21). GRP-irp may be present in nociceptive sensory neurons of either afferent vagal or dorsal root ganglion origin (5, 22). GRP-immunoreactive material has been identified in feline peritracheal ganglion cells (13) which may belong to the intrinsic nervous system of the tracheobronchial tree, or be parasympathetic post-ganglionic cell bodies.

The possible mechanisms that govern the release of GRP-irp into the pulmonary milieux are also diverse. Neuroendocrine cells may respond to chronic cigarette smoke by increasing GRP-irp content (3, 21). If GRP-irp are present in nociceptive sensory neurons (5, 14, 22), they may be released with other colocalized neuropeptides by axon response mechanisms. Axon reflexes have been shown to release substance P, neurokinin A and calcitonin gene related peptide from sensory neurons in rat nasal mucosa and rat tracheobronchial mucosa (23, 24). Axon reflexes may contribute to the pathogenesis of asthma (25). GRP-irp release by axon reflex mechanisms, or after capsaicin treatment, has not yet been examined. If present in ganglionic cells, then parasympathetic stimuli may induce the release of GRP-irp.

Based upon the autoradiographic data presented here, GRP or GRP-irp may be specific regulators of glandular secretion in human pulmonary mucosa. This finding may be of pathological importance in settings where increased amounts of GRP or GRP-irp are generated. Could the GRP-irp found in bronchoalveolar lavage fluid of cigarette smokers (3) play a role in mucus hypersection and chronic bronchitis? Given the limited density of binding sites on human vascular and bronchial smooth muscle, GRP likely has negligible direct effects on tracheobronchial smooth muscle tone and vascular tone or permeability.

Acknowledgements

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