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Occurrence and Distribution of GRP-Immunoreactive Nerve Fibres in the Respiratory Tract

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Summary. The occurrence and distribution of nerve fibres containing gastrin-releasing peptide (GRP) were investigated in the respiratory tract of several mammals using immunocytochemistry. A moderate supply of nerve fibres displaying GRP immunoreactivity was seen in the middle ear mucosa, the nasal mucosa and the tracheobronchial wall. Generally, the fibres were distributed around blood vessels and seromucous glands. In addition, scattered GRP fibres were seen in the smooth muscle of the tracheal wall. The distribution of GRP fibres in the respiratory tract suggests multiple functions of GRP such as regulation of local blood flow, glandular secretion and smooth muscle activity.

Key words: Gastrin releasing peptide – Respiratory tract – Immunocytochemistry

Introduction

A large number of biologically active peptides have been isolated from the skin of amphibians [9]. One such peptide is bombesin, which consists of 14 amino acid residues [1]. Bombesin was found to have potent biological actions in mammals such as release of gastrin and other gut hormones, stimulation of the exocrine pancreas and stimulation of gut smooth muscle [7, 10, 12, 14]. Radioimmunological and immunocytochemical studies have indicated the presence of bombesin-like immunoreactivity in the mammalian brain [3] and gut [8].

Recently, McDonald et al. [17] isolated a gastrin-releasing peptide [GRP] containing 27 amino acid residues from porcine gastric mucosa. The C-terminal region of GRP shows a striking homology with bombesin, nine out of 10 amino acid residues being identical. The biological activities of GRP are very similar to

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those of bombesin [18]. Thus, since authentic bombesin has not been isolated in mammals, it has been suggested that GRP is the mammalian counterpart of bombesin. This view is corroborated by the results of radioimmunological and immunocytochemical studies revealing immunoreactive GRP in the mammalian brain and gastro-intestinal tract [17, 19, 25].

The present study deals with the occurrence and distribution of GRP containing nerve fibres in the respiratory tract of several mammals.

Material and Methods

Cats, guinea-pigs, rats and mice were chosen for the experiments and at least five of each species were used. Cats were killed by exsanguination during pentobarbitone anaesthesia. Guinea-pigs, rats, and mice were killed by an overdose of diethylether and perfused via the heart with large volumes of ice-cold fixative (4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.2) for 10 min. From all animals, specimens were dissected out from the nose, larynx and trachea and immersed in the fixative for 8–12 h. The specimens were thoroughly rinsed in buffer containing 5%–25% sucrose for 48 h. They were then frozen on dry ice and sectioned (15 μm thickness) in a cryostat at −20° C. Fresh specimens from the guinea-pig middle ear mucosa, trachea, and main bronchi were stretched on chrome-alum coated microscope slides as whole mounts, fixed in a mixture of formaldehyde and picric acid, dehydrated in a series of ethanol solutions, cleared in xylene and hydrated [6].

The specimens were processed for the immunocytochemical demonstration of GRP using the indirect immunofluorescence method of Coons et al. [5] or the immunoperoxidase method of Sternberger [22].

The antiserum was raised in rabbits against synthetic GRP [25]. This antiserum is directed against the COOH-terminal portion of GRP and cross-reacts with bombesin. It does not cross-react with substance P with which GRP and bombesin share the two COOH-terminal amino acid residues. The antiserum was used in dilution 1 : 640 (for cryostat sections) and 1 : 320 (for whole mount preparations). The site of antigen-antibody reaction was revealed by fluorescein isothiocyanate labelled goat anti-rabbit IgG (DAKO Immunoglobulin AB, Stockholm, Sweden) and diluted 1 : 20. Some of the whole mounts were subsequently washed, incubated with a peroxidase-antiperoxidase complex and stained for peroxidase. Sections and whole mounts incubated with GRP antiserum inactivated by the addition of synthetic GRP served as controls [Beckman S.A., Geneva, Switzerland (10 μg/ml diluted antiserum)].

Cross reactivity with unknown peptides containing immunoreactive amino acid sequences recognized by the antiserum cannot be excluded. It would therefore be appropriate to name the immunoreactive material GRP-like. For brevity, the immunoreactive nerve fibres are referred to as GRP nerve fibres in the text.

Results

Nerve fibres displaying GRP immunoreactivity were detected in the respiratory tract of all species examined. On the whole, GRP fibres were moderate in number in cats and guinea-pigs whereas they were scarce in rats and mice.

Middle Ear

In the middle ear mucosa of cats and guinea-pigs, single fine-beaded GRP immunoreactive fibres were seen close to small blood vessels. In addition, certain fibres were seen to run in the mucosa with no obvious relation ship to the blood vessels (Fig. 1).

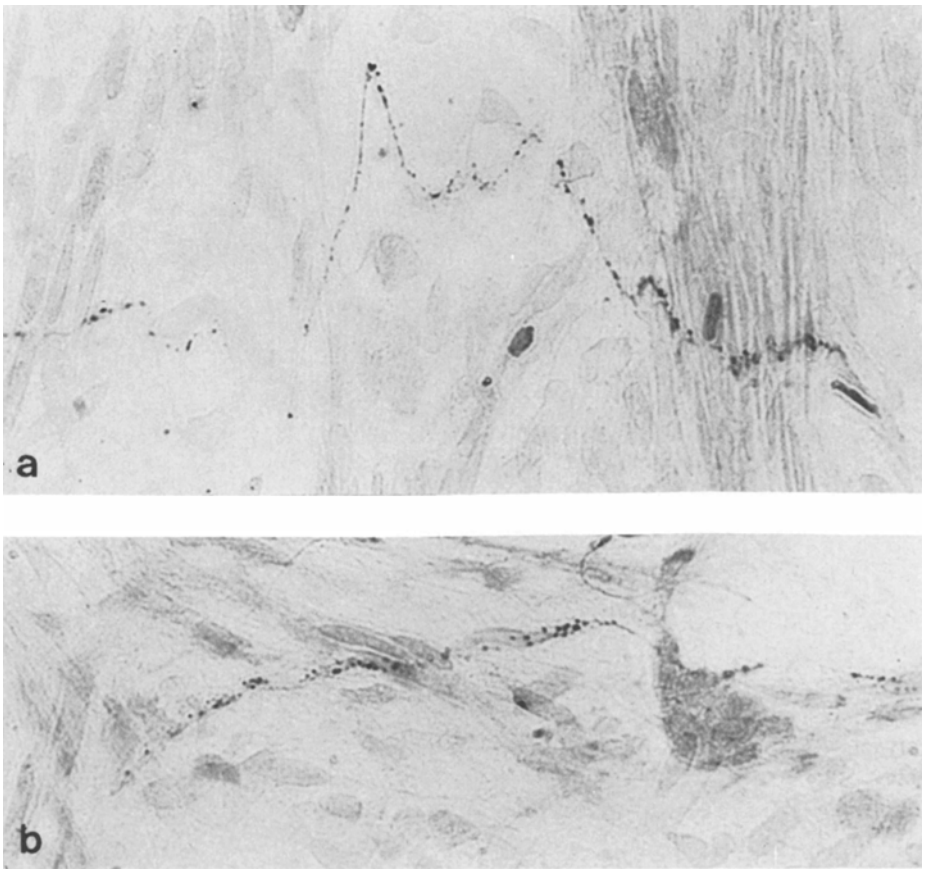

Fig. 1a and b. Guinea-pig middle ear mucosa × 250. **a** Single thin GRP immunoreactive nerve fibre. **b** A small nerve bundle containing several GRP immunoreactive nerve fibres

Nasal Mucosa

In the nasal mucosa of guinea-pigs and cats fine varicose GRP fibres were seen around seromucous glands and small blood vessels (Fig. 2). A moderate number of GRP fibres was seen in the maxilloturbinal area, whereas the fibres were scarce in the septum and in other parts of the nasal mucosa. GRP fibres were not seen in the nasal mucosa of rats and mice.

Tracheobronchial Wall

GRP fibres were seen in the tracheobronchial wall of all species examined. The fibres were more numerous in the upper parts of the tracheobronchial wall than in the lower parts. Fine varicose GRP fibres were seen in the smooth muscle layer (Fig. 3), around submucosal glands (Fig. 4) and around small blood vessels.

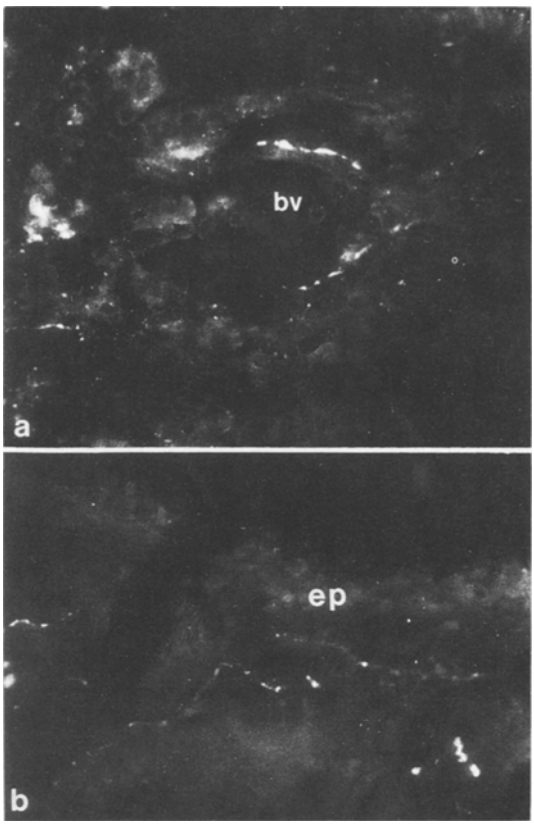

Fig. 2a and b. Guinea-pig nasal mucosa × 300. **a** GRP immunoreactive nerve fibre close to a small blood vessel (bv). **b** Thin GRP fibres in the subepithelial layer ep = epithelium

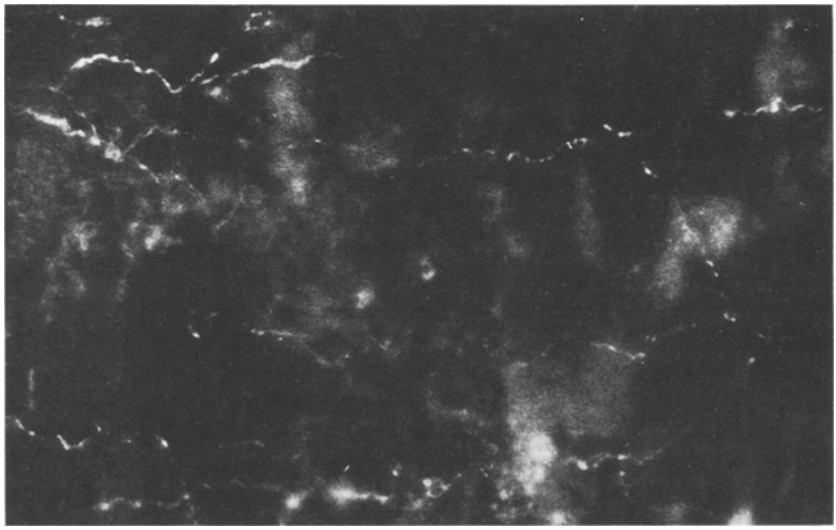

Fig. 3. Stretch preparation of the smooth muscle of the cat tracheal wall. Numerous thin GRP fibres are dispersed among the muscle bundles × 200

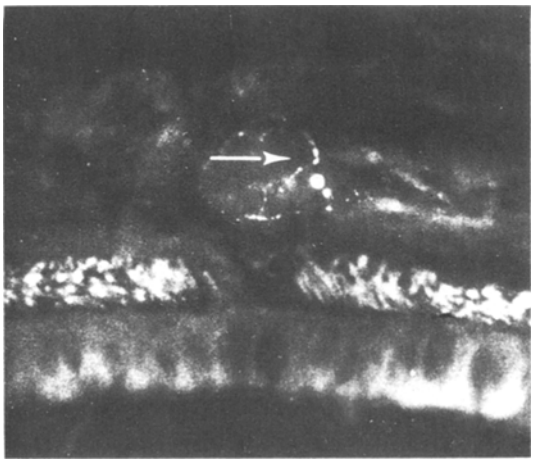

Fig. 4. Thin fine varicose GRP fibres (arrow) are seen around a sero-mucous gland in the subepithelial layer of the cat tracheal wall × 250

Discussion

The upper respiratory tract harbours a rich supply of autonomic nerve fibres. Besides the "classical" adrenergic and cholinergic (acetylcholinesterase-positive) nerves, several studies have described non-cholinergic, non-adrenergic components. The existence of peptidergic components in the autonomic nervous system has only recently been acknowledged. We have reported on the occurrence of nerve fibres containing vasoactive intestinal peptide (VIP) and substance P in the nasal mucosa and tracheo-bronchial tree [23, 24]. Both types of fibres are distributed around small blood vessels, sero-mucous glands and in the bronchial smooth muscle. Electrophysiological and pharmacological studies have also indicated the presence of non-cholinergic, non-adrenergic neuronal mechanisms in the regulation of tracheal smooth muscle tone and nasal blood flow [2, 4]. Recent studies have put forward VIP as a likely candidate for non-adrenergic relaxation of tracheal smooth muscle [16, 21] and for the atropin-resistant vasodilatation in the nasal mucosa [15]. Substance P causes an increase in tracheo-bronchial smooth muscle tone in vitro [20].

In the present study, GRP-containing nerve fibres were found in smooth muscle, around blood vessels, and around sero-mucous glands in the upper respiratory tract. Bombesin exerts a strong bronchoconstrictory effect in the guinea-pig in vivo [13]. The lack of inhibitory effects on this response that antagonists to several other putative transmitters display indicate that there is a direct action of bombesin on the smooth muscle. GRP and bombesin seem to have the same spectrum of biological actions [18] and since the whole biological activity of bombesin resides in its C-terminal nonapeptide region [11], it is conceivable that results obtained with bombesin are also valid for GRP. Studies that have so far been performed on the pharmacological actions of GRP support this assumption [18]. The distribution of GRP fibres in the upper respiratory tract suggests that GRP participates in the regulation of smooth muscle activity, local blood flow and seromucous secretion.

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