THE LOCALIZATION OF BASIC FIBROBLAST GROWTH FACTOR (FGF-2) IN RAT SUBMANDIBULAR GLANDS

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ABSTRACT

The immunohistochemical localization of basic fibroblast growth factor (FGF-2) in the submandibular glands of the rat was investigated by use of an antiserum to FGF-2. Nerve fiber bundles with FGF-2-immunoreactivity were found in association with interlobular ducts and blood vessels; they dissociated into single immunoreactive nerve fibers perhaps to terminate in proximity to acinar cells, or to form a reticular fiber network within the tunica adventitia of blood vessels. The FGF-2-immunoreactive neurons were located in the submandibular ganglia, but not in the superior cervical ganglia; hence, at least some of these immunoreactive nerve fibers probably come from the submandibular ganglia and are of parasympathetic origin. Most of the epithelial cells of the intercalated and collecting ducts showed notable FGF-2 immunoreactivity. The characteristic distribution of FGF-2 immunoreactivity in both the neuronal and epithelial tissues of the salivary glands suggests a role of this growth factor in complex physiological processes within the salivary glands.

Key Words: Basic fibroblast growth factor, Submandibular gland, Submandibular ganglion, Superior cervical ganglion, Immunohistochemistry

INTRODUCTION

Basic fibroblast growth factor (FGF-2) stimulates the mitogenic activities of a variety of mesoderm- and neuroectoderm-derived cells, as well as to facilitate the survival and differentiation of these cells. It has been detected biochemically in the retina, corpus luteum, adrenal gland, kidney, placenta, macrophages and prostate. Recent immunohistochemical studies showed FGF-2 localized not only in peripheral tissues but also in neuronal and neuroglial elements of the brain. In peripheral nerves, FGF-2 is abundant within the somatic motor and sensory nervous systems. However, there are few studies on FGF-2 in the peripheral autonomic nervous system.

The secretory activity of the salivary glands is controlled by the autonomic nerves. It has been proposed that parasympathetic nerve fibers regulate the viability of glandular cells, because their transection or interruption produces degeneration of the acinar cells. We sought to determine whether FGF-2, like nerve growth factor, is localized to the salivary glands and/or to the parasympathetic nerves that innervate the glands. Using a FGF-2 antiserum that has been characterized by immunoblot, we investigated the distribution of FGF-2-like immunoreactivity in rat salivary glands with special attention given to the autonomic nerves.

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143
MATERIALS AND METHODS

Animals

Ten male Sprague-Dawley rats weighing 100 to 150 g were used in this study. The animals were kept on a 12h:12h light-dark cycle, and given food and water ad libitum. The following experiments were conducted in accordance with the Guide for Animal Experimentation at Nagoya University School of Medicine.

Tissue preparation

The animals were anesthetized with pentobarbital (40 mg/kg) injected into the abdominal space and perfused transcardially, first with 100 ml of saline, then with 200 ml of fixative consisting of 4% paraformaldehyde and 0.2% picric acid in 0.1M phosphate buffer (PB) (pH 7.4). After perfusion, the submandibular gland, sublingual gland, superior cervical ganglion, and submandibular ganglion were excised and postfixed overnight with the same fixative at 4°C. The tissues were then immersed overnight in 0.1M PB containing 30% sucrose at 4°C and cut into 10-μm sections in a cryostat. The sections were mounted on gelatin-coated slides.

Preparation of polyclonal antibody to FGF-2

In order to affinity-purify anti-FGF-2 IgG, 200 μg of FGF-2 in a partially purified fraction was separated by SDS-PAGE and transblotted to nitrocellulose membranes. FGF-2 bands were excised as small pieces and treated with 5% bovine serum albumin (BSA) in phosphate buffered saline (PBS) overnight at 4°C to block nonspecific binding of antibody. Then the protein A purified IgG fraction of anti-FGF-2 was added to the excised blots and incubated overnight at 4°C with end-over-end mixing. After washing with PBS ten times, antibody bound to FGF-2 was eluted with 0.2M glycine-HCl, pH 3.0, 0.15M NaCl. The purification steps were repeated several times using excised blots washed with PBS. The final eluted solution was immediately neutralized, supplemented with BSA to 1 mg/ml and dialyzed with PBS containing 0.02% sodium azide.

Immunohistochemical procedures

Sections were processed for immunohistochemistry with a FGF-2 antiserum that had been characterized by Western blot analysis elsewhere.

Briefly, the sections were 1) incubated for 48 h with FGF-2 antiserum, diluted 1:1000 with 0.1M PBS containing 5% BSA, 1% normal goat serum (NGS), 0.1% Triton X-100 (TX), and 0.1% sodium azide; 2) washed three times with 0.1M PBS containing 1% NGS, (10 min for each washing); 3) incubated overnight with biotinylated anti-rabbit goat IgG (VECTOR, Burlingame, U.S.A.) diluted 1:250 with the same solution; 4) washed three times with 0.1M PBS containing 1% NGS, (10 min for each washing); 5) incubated for 24 h with peroxidase-conjugated streptavidin (Kirkegaard & Perry Lab, Inc., Gaithersburg, U.S.A.), diluted 1:300 with 0.1M PBS containing 5% BSA and 0.1% TX; 6) washed twice with 0.1M PBS, once with 0.05M Tris-HCl buffer (TB) (pH 7.4) and finally with 0.1M TB for 10 min each; and 7) subjected to a modified version of the cobalt-glucose oxidase-diaminobenzidine intensification method. After immunostaining, the sections were dehydrated in a graded series of ethanol and coverslipped. Control sections were incubated with the antiserum that had been adsorbed with bovine FGF-2 and processed as described above.
RESULTS

Nerve fibers with FGF-2-like immunoreactivity ran between the submandibular and sublingual glands and entered the cranial apexes of the glands. They gave rise to branches into the interlobular spaces running along local arteries or glandular ducts (Fig. 1a, b). No positive reactions occurred in preadsorption control sections (Fig. 1c, d). In the more peripheral regions of the glands, FGF-2-positive nerve fibers decreased in number; occasionally, a few immunoreactive nerve fibers were located close to acinar cells of the submandibular gland (Fig. 2a, b).

![Fig. 1. Bright-field photomicrographs showing, at low (a) and high (b) magnification, the main trunk of FGF-2-immunoreactive nerve fibers in an interglandular space (arrowheads). a, ×100; b, ×400 Bright-field photomicrographs showing, at low (c) and high (d) magnification, a preadsorption control section. c, ×100; d, ×400. The positive reaction, as seen in Fig. 1a, b, is abolished (arrowhead).]
To deduce the origin of FGF-2-immunoreactive fibers in the salivary glands, the submandibular and superior cervical ganglia were immunostained. The submandibular ganglion, which was located in the connective tissue between the submandibular and sublingual glands, contained immunoreactive ganglion cells (Fig. 3a). Immunoreaction products were seen mainly in the cytoplasm, but rarely in the nuclei of the ganglion cells. In preadsorption control sections, these reactions were eliminated (Fig. 3b). There were no reactions in the superior cervical ganglion (Fig. 3c).

Fig. 2. Bright-field photomicrographs showing nerve fibers with FGF-2 immuno-reactivity (arrowheads) in the submandibular gland. Some run from an interlobular space to an adjacent lobule (a), and others are located close to acini (b). a, ×400; b, ×400
Intense FGF-2-positive reactions were noted in the epithelial cells of collecting and intercalated ducts; striated duct epithelium and acinar cells exhibited less intense immunoreactions (Fig. 4a, b, c, d, e). Many FGF-2-immunoreactive nerve fiber bundles were localized to the walls of arteries (Fig. 5a, b) and dissociated into fine branches that formed a network on the surface of the tunica adventitia (Fig. 5c). Such FGF-2-positive reactions were also found in the endothelial cells of relatively large arteries and veins within the salivary glands (Fig. 5d). In preadsorption control sections, there were no reactions in the epithelial cells of ducts and blood vessels (Fig. 4f).
Fig. 4. Bright-field photomicrographs showing, at low (a) and high (b) magnification, a FGF-2-immunopositive collecting duct (double arrowheads). Arrowhead indicates FGF-2-immunoreactive nerve fibers subjacent to the duct. a, ×100; b, ×400

Bright-field photomicrographs showing FGF-2-positive reactions in the epithelia of a relatively small interlobular duct (double arrowheads) (c), a striated duct (double arrowheads) (d), and an intercalated duct (double arrowheads) (e). c, ×400; d, ×400; e, ×200

Fig. 4f. Bright-field photomicrographs showing disappearance of immunoreaction in a preadsorption control section: collecting duct (arrow head), striated duct (double arrowhead) and blood vessels (open arrowhead) ×200
DISCUSSION

The present study demonstrated localization of FGF-2 in putative autonomic nerves. The presence of FGF-2-immunopositive ganglion cells in the submandibular ganglion, but not in the superior cervical ganglion, suggests that some of the immunoreactive nerve fibers in the salivary glands are of parasympathetic origin. It is likely that immunoreactive nerve fibers in the walls of arteries are derived from sensory ganglia. A recent (in vitro) experiment showed that FGF-2 facilitates the survival and development of cholinergic neurons and fibers. Furthermore, parasympathetic denervation was reported to cause hypofunction and degeneration of the salivary glands in rats. Our results, together with the above experiments, show that FGF-2 may participate in the maintenance or trophism of rat salivary glands.

The presence of FGF-2-immunoreactivity in the epithelia of collecting and striated ducts raises the question of whether the growth factor is secreted into the lumen and/or adluminal
spaces, because most members of the FGF family including FGF-1 and FGF-2 are devoid of signal sequence. Mignatti et al. say that FGF-2 is released from the cell surface in a novel exocytic way independent of the classic endoplasmic reticulum-Golgi complex route and possibly binds to heparan-sulfate proteoglycan in the extracellular matrix. Although the FGF-2 antiserum used in the present study may not have recognized FGF-2 molecules bound with heparan-sulfate in the extracellular matrix, DiMario et al. and Gonzalez et al. reported that the antisera they used reacted with them. Thus, we could not determine whether bFGF is secreted in the salivary glands. Use of antibodies that are directed to heparan-sulfate-bound FGF-2 may clarify this uncertainty.

The role of high concentration FGF-2 molecules in the intercalated ducts, as revealed by the present immunohistochemical study, is not clear. However, the characteristic distribution of FGF-2 immunoreactivity suggests its important role in the physiological process.

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REFERENCES

BASIC FIBROBLAST GROWTH FACTOR (FGF-2)


