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EXPRESSION OF NERVE GROWTH FACTOR IN RAT STOMACH. IMPLICATIONS FOR INTERACTIONS BETWEEN ENDOTHELIAL, NEURAL AND EPITHELIAL CELLS

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This study was aimed to determine the expression and localization of nerve growth factor (NGF) in the gastric mucosa. Transmural gastric specimens were obtained from euthanized rats. Studies: 1) expression of NGF and TrkA receptor by Western blotting; 2) histological evaluation of gastric wall architecture; 3) expression of NGF using immunostaining. Immunostaining showed strong and differential expression of NGF in neural elements of gastric myenteric and submucosal plexuses; in epithelial cells: mainly in chief and progenitor cells, in enterochromaf fin-like (ECL) cells; and, in endothelial cells (ECs) lining blood vessels. We concluded that NGF expression in neural elements, epithelial cells and endothelial cells of blood vessels indicated a complex local interaction between neural, epithelial and endothelial cells that regulated gastric mucosal homeostasis and, likely, the protection against gastric injury and ulcer healing.

Key words: enteric nervous system, stomach, nerve growth factor, Trk receptor, chief cells, endothelial cells, epithelial cells, afferent neurons

INTRODUCTION

Nerve growth factor (NGF) was discovered in the early 1950s by Rita Levi-Montalcini who, together with Stanley Cohen, shared the Nobel Prize in 1986 for the discovery of NGF and epidermal growth factor (EGF). They demonstrated how the growth and differentiation of cells and tissues are regulated (1-3). The discovery of NGF opened new fields of basic science and had profound clinical implications.

NGF has been shown to be present in (and secreted by) malignant mouse sarcoma cells, snake venom secreting glands, salivary glands of rodents (1-4) and humans (5). Studies in the early 1970s identified the structure of NGF, which led to the discovery of NGF gene. The NGF precursor, also referred to as pro-NGF, is a complex 130 kDa protein consisting of α-NGF, β-NGF, and γ-NGF subunits (6). The γ-subunit, acting as a serine protease, cleaves the N-terminal of the β-NGF subunit and activates the protein into the functional NGF. The term NGF usually refers to the 26 kDa β-subunit of the protein that is the biologically active ligand for its receptors. The active β-NGF is a dimer of two identical 13 kDa subunits held together by strong hydrophobic interactions (6, 7).

NGF exerts a variety of actions on neurons of the central nervous system, including the regulation of proliferation, differentiation, neurite outgrowth, neuro-transmission, plasticity, repair, and survival (1, 2, 5). NGF has been shown to be critical for the survival and preservation of sympathetic and sensory neurons, axonal growth and branching. NGF binding to its high-affinity tyrosine kinase receptor, TrkA, initiates TrkA phosphorylation, which triggers activation of the PI-3 kinase, ras and PLC signaling pathways. NGF also binds to the p75 neurotrophin receptor (p75NR), which exhibits low affinity for NGF (1, 2, 5, 6).

NGF prevents or reduces neuronal degeneration in animal models of neuro-degenerative diseases; and, importantly, had been proven effective in human clinical trials (8, 9). NGF promotes peripheral nerve regeneration in rats (10) by promoting myelin repair (11). Human studies demonstrated that NGF may be beneficial for the treatment of multiple sclerosis, and Alzheimer’s disease (8, 9, 11-14).

Until recently, the roles of NGF have been almost exclusively investigated within the contexts of differentiation, growth and survival of specific neurons in the peripheral and central nervous system. However, in the last decade several non-neuronal functions of NGF and other neurotrophic factors have been characterized. In the gastrointestinal tract, neurotrophic factors regulate neuropeptide expression, interact with immune regulatory cells and epithelial cells; and, regulate motility during inflammation (15-20). These findings highlight a new and complex regulatory system that may lead to new therapeutic approach to the treatment of acute and chronic gut inflammation.

Some studies indicate that, in addition to affecting neurons, NGF may play a critical role in the maintenance and survival of pancreatic beta cells (21) and in regulation of both innate and acquired immunity (22). NGF is produced by the thymus as well as CD4+ T cell clones, inducing a cascade of T cell maturation during infection. NGF may also play a role in inflammation as it is released in high concentrations by mast cells and induces axonal outgrowth in adjacent nociceptive neurons leading to increased pain perception in inflamed tissues (23). NGF may play a role in gastric cancer but this remains controversial. Du et al. (24)
showed that expression of NGF and Trk receptor is downregulated in gastric carcinoma comparing with normal gastric mucosa while Hayakawa et al. suggest that NGF may promote gastric tumorigenesis through aberrant cholinergic signaling (25).

NGF has been shown to play a role in a number of cardiovascular diseases, such as coronary atherosclerosis, obesity, type 2 diabetes, and metabolic syndrome (22, 26, 27). Reduced plasma levels of NGF and BDNF have been associated with acute coronary syndromes and metabolic syndromes (26, 28). NGF is known to have insulinotropic, angiogenic, and antioxidant properties (29, 30). NGF has also been shown to accelerate wound healing (31, 32). There is evidence that it could be useful in the treatment of skin ulcers and corneal ulcers (33, 34).

Expression of NGF in the stomach has not been examined in details before. Bielefeldt et al. (35) demonstrated the expression of NGF in inflamed gastric mucosa of rats, but not in normal gastric mucosa, and, examined the role of NGF in the modulation of gastric afferent neurons in the rat stomach. They provided evidence that inflammation increased the expression of NGF within the gastric wall which persisted for more than 2 weeks al (36). This group of investigators has also shown that increased NGF contributes to the development of hyperalgesia after gastric injury (36).

In the present study we focused on expression of NGF and TrkA receptors in normal stomach of rats.

**MATERIAL AND METHODS**

The study was approved by the institutional animal review committees: Subcommittees for Animal Studies (IACUC), of the Veterans Affairs Long Beach Healthcare System, California, USA, and the Jagiellonian University Medical College, Cracow, Poland and performed in accordance of Helsinki Declaration regarding handling of experimental animals.

**Animal studies**

We performed studies in 15 *H. pylori* and viral free Fisher F-344 male rats 3 months of age purchased from the National Institute on Aging, Bethesda, MD, USA. Rats were euthanized and gastric wall specimens were obtained and fixed in 10% buffered formalin for histology and immunohistochemistry. Specimens were embedded in paraffin and routinely processed and stained with hematoxylin and eosin. For the molecular biology studies, gastric specimens were frozen at −70°C.

**Assessment of nerve growth factor and TrkA receptors in gastric mucosa by Western blotting**

Rat gastric mucosal specimens frozen at −70°C were homogenized with a Polytron homogenizer in lysis buffer and processed routinely for Western Blotting as described in our previous studies (37). Expression of NGF and TrkA was determined by Western blotting using respective specific antibodies and normalized using β-actin as an internal control reference. The primary antibodies used for these studies were NGF (1:250, sc 548, Santa Cruz Biotechnology, Santa Cruz, CA), TrkA (1:250, sc 14024, Santa Cruz Biotechnology, Santa Cruz, CA) or β-actin (1:1000, Sigma, MO).

**Determination of nerve growth factor and TrkA receptors by immunostaining in gastric wall of the stomach**

Full thickness sections of gastric walls were immunostained with specific antibodies against NGF (sc 548; Santa Cruz Biotechnology, Santa Cruz, CA) and TrkA (sc 118; Santa Cruz Biotechnology, CA) using methods previously described in our studies (37).

**RESULTS**

Western blotting studies for NGF and TrkA showed strong bands at ~13 kDa and 140 kDa, respectively demonstrating expression of NGF and its TrkA receptor proteins in gastric specimens (Fig. 1).

As shown in Fig. 2, the immunostaining demonstrated a strong expression of NGF in neural elements of gastric myenteric and submucosal plexuses; in epithelial cells (mainly in chief and progenitor cells) and endothelial cells (A), the enterochromaffin-like (ECL) cells (B), the gastric mucosal and myenteric plexuses (C and D, respectively), and, in the endothelial cells (ECs) lining blood vessels (E). The negative control of the gastric mucosal tissue immunostained without NGF antibody is presented in (F).

**DISCUSSION**

This study showed for the first time expression and localization of NGF in normal (non-cancerous, non-ulcerated) gastric mucosal tissue. A previous study detected NGF expression in inflamed gastric mucosa of rats but not in normal gastric mucosa (35). The difference between that study and the present study may be due to different fixation and processing techniques and/or the use of antigen retrieval in our study.

Our previous studies showed expression of NGF in porcine esophagus and in myenteric and submucosal plexuses of porcine stomach (38, 39). Demonstration in the present study of NGF expression in neural elements of gastric myenteric and submucosal plexuses, in epithelial cells, (ECL) cells and ECs of blood vessels indicates important potential local interactions that are required for the regulation of these cellular components. Expression of NGF in gastric ECs indicates that NGF may regulate these cells via circulating blood as well as via local autocrine action. Our recent study demonstrated that NGF is expressed in gastric ECs and is a major factor regulating angiogenesis and EC proliferation (40, 41). Originally, NGF has been shown to enhance the gastric mucosal defense and protect gastric mucosa against ethanol-induced gastric

**Western blotting for NGF and TrkA in rat gastric mucosa**

NGF (13 kDa)  
TrkA (140 kDa)  
β-actin

Fig. 1. Western blotting for NGF and its receptor TrkA in rat gastric mucosa. NGF and TrkA are expressed in normal rat gastric tissue.
mucosal injury (42). The potential gastroprotective and pro-healing ability of NGF might open new possibilities for this growth factor in modulating both, the cell protection and healing processes in several pathological conditions such as corneal ulcers, pressure ulcers, post-viral infections, and chemical burns in which it might shorten the recovery process (33, 34). Our preliminary study demonstrated for the first time that during gastric ulcer healing locally administered exogenous nerve growth factor is retained in gastric tissue and is taken up by endothelial, neural, muscle and epithelial cells (43). This is likely the basis for the therapeutic action of locally administered nerve growth factor and its stimulation of angiogenesis, tissue regeneration and gastric ulcer healing (43). Indeed, this therapeutic efficacy of neurotrophins including NGF, brain derived neurotrophic factor (BNDF), NT-3 and NT-4 associated with an expression of TrkA receptors have recently been implicated in the mechanism of neuronal protection and stem cells-based regenerative therapy exhibited by bone marrow cells (44).

Conflict of interests: None declared.

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