Role of Neurotrophins and Neurotrophin Receptors in Rat Conjunctival Goblet Cell Secretion and Proliferation

J. David Rios, Emiliano Gbinelli, Jian Gu, Robin R. Hodges, and Darlene A. Dart

PURPOSE. To determine which neurotrophins (NTs)—nerve growth factor (NGF), brain-derived neurotrophin (BDNF), neurotrophin-3 (NT3), and neurotrophin-4 (NT4)—and their receptors (NTrs) TrkA, TrkB, TrkC, and p75 are present in rat conjunctiva and cultured rat goblet cells (CGCs) and whether NTs stimulate glycoconjugate secretion or cell proliferation.

METHODS. Western blot analysis and immunofluorescence microscopy determined presence and location of NTs and NTrs. CGCs were incubated with NTs (10–12–10–8 M) for 2 or 24 hours to measure secretion or proliferation, respectively. An enzyme-linked lectin assay analyzed glycoconjugate secretion. WST-8 determined cell proliferation.

RESULTS. Western blot analysis showed all NTs and NTrs in both conjunctiva and CGCs. The cytoplasm of conjunctival stratified squamous cells and goblet cell lateral membranes contained NGF, BDNF, and NT4. Stratified squamous cell membranes contained NT3. In CGCs, NGF and BDNF had punctuate perinuclear staining. The nucleus contained NT3 and cytosol contained NT4. TrkA, TrkB, and p75 immunoreactivity was on conjunctival goblet cell lateral membranes. Plasma membranes of the basal layer of stratified squamous cells contained TrkA. Stratified squamous cell and goblet cell nuclei contained TrkC. In CGCs, all NTrs were present in the nucleus. NGF and BDNF, but not NT3 and NT4, induced a concentration-dependent stimulation of secretion from CGCs with a maximum increase of 10–9 M each. No effect on cell proliferation was detected with any NTs.

CONCLUSIONS. Rat conjunctival goblet cells and CGCs contain all NTs and NTrs. Only NGF and BDNF stimulated goblet cell glycoconjugate secretion, and none induced CGC proliferation. (Invest Ophthalmol Vis Sci. 2007;48:1543–1551) DOI:10.1167/iovs.06-1226

 Conjunctival goblet cells are the primary source of the large soluble mucins in the tear film. These mucins provide a physical and chemical barrier that protects the cornea and conjunctiva from exogenous agents (bacterial or chemical) and facilitates the occurrence of a smooth refractive surface necessary for clear vision. Goblet cells release their secretory granules in a response mediated by the reflex activation of the parasympathetic nerves that surround them. Neurotransmitters such as acetylcholine and the neuropeptide vasoactive intestinal peptide (VIP) are well-known stimuli of conjunctival goblet cell mucin secretion and the respective receptors and signaling pathways activated by these stimuli have been characterized.1 Neurotrophins are a family of dimeric proteins that regulate the proliferation, survival, and differentiation of neurons in all vertebrate species. Four members of this family have been identified: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins-3 (NT3) and -4 (NT4).2 These compounds have similar physical characteristics and are synthesized as high-molecular-weight precursors that are cleaved to form the mature protein3–5 and exist as dimers. In addition, these proteins can be glycosylated and glycosulfated, which alters their molecular weight.

Three main full-length high-affinity neurotrophin receptors (NTrs) have been discovered and are known as the tyrosine kinase (Trk) receptors TrkA, TrkB, and TrkC.6 In addition, a non-Trk receptor p75 can bind each NT. The p75 receptor is a member of the tumor necrosis factor family and regulates the affinity of the Trk receptors for their NTs.7 The NTrs show high binding affinity for the following NTs: NGF preferentially binds to TrkA, but NT-3 and NT-4 can also bind to TrkA.8,9 BDNF and NT4 can bind to TrkB, whereas NT-3 preferentially binds to TrkC.6,10

Neurotrophins and their receptors are also expressed in a variety of non-neuronal cell types.11,12 In addition to neurotrophins’ general roles as growth and survival factors in the nervous system, changes in their expression have been associated with physiological or pathologic processes, such as activation, proliferation, or repair after injury in non-neuronal tissues.13–15 Neurotrophins have also been involved in various inflammatory and noninflammatory disorders.12 Several studies suggest that NGF stimulates epithelial cell proliferation. On the ocular surface, NTs and their receptors have been identified in tears, cornea, lens, and lacrimal glands,16–20 suggesting a potential role in regulating the functioning of the ocular surface through its receptors. An increased level of NGF in tears has been reported in keratoconjunctivitis sicca, and after corneal epithelial wounding in an animal model,16,21 which suggests the involvement of NGF in the conjunctival inflammatory process and in corneal epithelial proliferation and differentiation.22 Several clinical studies have demonstrated that topical NGF treatment improves the corneal sensitivity and corneal epithelial healing in both moderate and severe neurotrophic keratitis, suggesting that NGF modulates corneal healing in neurotrophic corneal disease.22 In addition, a recent study showed that topical NGF treatment improves the ocular surface of dogs with surgically induced dry eye.21 In this study, there was a significant increase in conjunctival goblet cell density. The effect of NGF on the number of conjunctival goblet cells suggests that NGF might modulate the proliferation of these cells through its receptors.

There is no evidence to date that supports the role of neurotrophins in goblet cell secretion. Therefore, the present study was designed to determine the expression and localization of NTs and NTrs in goblet cells in situ in the conjunctiva and in culture. We also investigated the effect of the neurotrophins and their receptors on the regulation of conjunctival goblet cell glycoconjugate secretion and proliferation.
MATERIALS AND METHODS

Recombinant human neurotrophins NGF, BDNF, NT-3, and NT-4 were purchased from Peprotech, Inc. (Rocky Hill, NJ). Antibodies to NTs and NTRs, their corresponding peptides, and the secondary antibody horseradish peroxidase (HRP)-conjugated IgG were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The chemiluminescence reagents for visualization of the blots were from Pierce (Rockford, IL). Fluorescein isothiocyanate (FITC)-conjugated lectin Ulex europaeus agglutinin I (UEA-I) was obtained from Eugene Tech International, Inc. (Ridgefield Park, NJ). Biotinylated UEA-I and streptavidin-conjugated alkaline phosphatase were obtained from Pierce (Rockford, IL). The rhodamine-conjugated secondary antibodies donkey anti-mouse and anti-rabbit IgG and FITC-conjugated donkey anti-mouse and anti-rabbit IgG secondary antibodies were purchased from Jackson ImmunoResearch (West Grove, PA). Mounting medium containing 4',6-diamidino-2-phenylindole (DAPI; Vectashield) was purchased from Vector Laboratories, Inc. (Burlingame, CA). RPMI 1640 culture medium, t-glutamine, and penicillin/streptomycin were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals were from Sigma-Aldrich (St. Louis, MO).

Animals

The use of animals conformed to the guidelines established by the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the Schepens Eye Research Institute Animal Care and Use Committee. Male Sprague-Dawley rats weighing between 250 and 300 g were used in the study and were obtained from Taconic Farms (Germantown, NY). The animals were anesthetized for 1 minute in carbon dioxide, decapitated, and the conjunctiva dissected.

Immunohistochemistry

For immunofluorescence microscopy, the eyes were enucleated with the lids intact and fixed in 4% formaldehyde in phosphate-buffered saline (PBS; 145 mM NaCl, 7.3 mM NaHPO₄, and 2.7 mM NaH₂PO₄ [pH 7.2]), for 4 hours at 4°C. The eyes were then rinsed in 5% sucrose dissolved in PBS, placed overnight in 30% sucrose dissolved in PBS at 4°C, embedded in OCT, and frozen. Cryostat sections (6 μm) were placed on slides (Colorfrost/Plus; Fisher Scientific, Pittsburgh, PA) and kept at −20°C until use. Cultured goblet cells (CGCs) grown on glass coverslips were fixed in absolute methanol. Tissue sections and CGCs were then washed in PBS and blocked in PBS containing 1% bovine serum albumin, 4% goat serum, and 0.2% to 0.3% Triton X-100. The NT antibodies (NGF, BDNF, NT3, and NT4) were used at a dilution of 1:800. The NTR antibodies (TrkA, TrkB, TrkC, and p75) were used at a dilution of 1:200. All antibodies were diluted in PBS containing 0.3% Triton X-100 and incubated for 48 hours at 4°C. The FITC-conjugated secondary antibody was then added (1:200) for 1 hour at room temperature. Tissue sections and CGCs were double-labeled with UEA-I, which indicates secretory granules in goblet cells and aids in the identification of goblet cells and with mounting medium containing 4',6-diamidino-2-phenylindole (DAPI), to identify cell nuclei. The slides were viewed and photographed with a microscope (UXF II Eclipse E800; Nikon, Melville, NY) equipped for epi-illumination microscopy and a digital camera (Spot; Diagnostic Instruments, Inc., Sterling Heights, MI). Negative control experiments included the omission of the primary antibody.

Western Blot Analysis

Pieces of rat conjunctiva and CGCs were homogenized in RIPA buffer (10 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% deoxycholic acid, 1% Triton X-100, 0.1% SDS, and 1 mM EDTA), containing protease inhibitors (10 μg/ml phenylmethylsulfonyl fluoride, 5 U/ml aprotinin, and 100 nM sodium orthovanadate). After homogenization, the samples were centrifuged at 2000 g for 30 minutes at 4°C. To detect NTs, proteins in the supernatant were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 15% acrylamide gels for NT. To detect the NTRs, membrane fractions were isolated from rat conjunctiva, CGCs, and brain homogenates prepared in homogenization buffer (30 mM Tris-HCl [pH 7.5], 10 mM EGTA, 5 mM EDTA, 1 mM dithiothreitol, and 250 mM sucrose) containing protease inhibitors (10 μg/ml phenylmethylsulfonyl fluoride, 5 U/ml aprotinin, and 1 mM sodium orthovanadate). After homogenization, the samples were centrifuged at 2000 g for 15 minutes at 4°C. The supernatant was centrifuged at 100,000 g for 1 hour at 4°C. The pellet was resuspended in the homogenization buffer and proteins separated by SDS-PAGE on 10% acrylamide gels.

![Figure 1](https://example.com/figure1.png)
Proteins were then transferred by electrophoresis to nitrocellulose membranes, blocked in 5% dried milk in TBST (10 mM Tris-HCl [pH 8.0]), 150 mM NaCl, and 0.05% Tween-20, and incubated with the indicated antibodies (1:400) overnight at 4°C. Membranes were washed three times in TBST and incubated with HRP-conjugated anti-rabbit IgG (1:2000) for 1 hour at room temperature. Immunoreactive bands were visualized by the enhanced chemiluminescence method. Rat brain homogenate was used as a positive control. Negative control experiments included the omission of the primary antibody.

Cell Culture

Explant cultures were established from rat inferior conjunctival tissue. Cells derived from the explants were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and penicillin (100 U/mL)/streptomycin (100 μg/mL) at 37°C in a humidified 5% CO₂ atmosphere for 72 hours. Contaminating nongoblet cells were removed by scraping them from the dish. During this time, goblet cells migrated from the pieces and began to proliferate. After 1 week, the goblet cells were trypsinized and plated in either 24-well culture plates or coverslips (for immunohistochemical study) with RPMI-1640 media supplemented with 10% FBS. To measure cell secretion, goblet cells were grown to confluence and serum depleted for 2 hours before stimulation. To measure cell proliferation, goblet cells were grown to subconfluence and serum deprived for 24 hours.

Measurement of Glycoconjugate Secretion

Cell were incubated with or without increasing concentrations of NTs (10⁻¹²–10⁻⁸ M), in serum-free RPMI supplemented with 0.5% BSA for 2 hours. The cholinergic agonist carbachol at 10⁻⁴ M was used as the positive control. Goblet cell secretion was measured using an enzyme-linked lectin assay (ELLA). The media were collected and analyzed for amount of the lectin-detectable glycoconjugates. These glycoconjugates include mucins and indicate goblet cell secretion. The amount of secretion was measured by using the lectin UEA-I which is specific for rat conjunctival goblet cell mucins. Bovine submaxillary mucin was used for the standard curve. Glycoconjugate secretion was expressed as the increase (x-fold) over basal.

Measurement of Cell Proliferation

Cells were incubated, with or without increasing concentrations of NTs (10⁻¹²–10⁻⁸ M), in serum-free RPMI supplemented with 0.5% BSA as a protein source, for 24 hours. RPMI supplemented with 10% FBS was used as the positive control in cell proliferation studies. CGC
proliferation was determined with a colorimetric nonradioactive, WST-8 proliferation assay that measures the number of cells. This procedure employs the 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-8), which is cleaved by viable, growing mitochondria to form a dark blue formazan product that is detected by a fluorescence ELISA reader (Bio-Tek, Winooski, VT) at 460 nm.

Data Presentation and Statistical Analysis

Data for CGC secretion and proliferation are expressed as the increase (x-fold) above basal value, which was standardized to 1.0. Results are expressed as the mean ± SEM. Data are analyzed by Student’s t-test. P < 0.05 is considered statistically significant.

RESULTS

Expression of Neurotrophin Protein in Conjunctiva and CGCs

The presence of each NT was determined in conjunctival tissue, CGCs, lacrimal gland, and brain, (positive control) by Western blot analysis (Fig. 1). Neurotrophins can be detected at multiple molecular weights, as they are synthesized as proforms that include a signal peptide and the mature molecule. In addition, they are often present in tissues as dimers and can be glycosylated on multiple sites.5,24,25 Pro-NGF has a molecular weight of 34 kDa, whereas the mature form has a molecular weight of 14 kDa. In brain, a positive control, the major NGF band had an approximate molecular weight of 40 kDa, which could indicate a glycosylated form of pro-NGF (Fig. 1A). In lacrimal gland (another positive control), homogenates from the conjunctiva and CGCs, major bands at approximately 30 and 25 kDa were detected. Lacrimal gland had an additional band ~14 kDa that could represent the mature form of NGF. Thus, in the conjunctiva and CGCs, NGF appears to be pro-NGF (Fig. 1A).

BDNF is also synthesized as an ~32 kDa form, whereas the molecular weight of the mature form is 14 kDa.26 In brain and lacrimal gland, the positive controls, bands were present at approximately 42, 35, and 28 kDa, with additional bands at approximately 17 and 13 kDa (Fig. 1B). In the conjunctiva and CGCs, three major bands were present at approximately 42, 35, and 13 kDa. In the conjunctiva and CGCs, BDNF appeared to be present in both the pro- and mature form and could be present in a glycosylated form (Fig. 1B).

The monomer of NT3 has a molecular mass of 13 kDa, whereas the dimer has a molecular mass of 27 kDa. In brain
and lacrimal glands, bands were present at 48, 35, and 27 kDa. The conjunctiva and CGCs had one major band approximately 35 kDa (Fig. 1C) that could be a pro form or a glycosylated form.

NT4 has a molecular mass similar to that of NT3. In all samples, several bands were present clustered between 30 and 45 kDa and a single band at approximately 20 kDa (Fig. 1D). These multiple bands at 45 kDa could represent differentially glycosylated, NT4 and the band at 20 kDa could be a monomer.

**Immunolocalization of Neurotrophins in Conjunctiva and CGCs**

In the rat conjunctiva, goblet cells are present in clusters and are located in the apical surface interspersed among multiple layers of stratified epithelium. Goblet cells are easily recognized by their vast accumulation of secretory vesicles containing mucins that bind to the lectin UEA-1. Goblet cell bodies are
subjacent to the secretory granules and extend to the basement membrane.

As shown in Figure 2, goblet cells were identified by the presence of UEA-1 staining (shown in red) in rat conjunctival sections. Positive immunofluorescence staining for all four NTs (shown in green) was observed in the lateral membranes and cytoplasm of goblet cells in rat conjunctiva. A punctate cytoplasmic staining pattern was observed in the conjunctival stratified squamous cells for NGF, BDNF, NT3, and NT4. NT3 was also localized to membranes of stratified squamous cells. Punctate staining for each NT was also obtained in the conjunctival stroma (Fig. 2). In conjunctival sections, neurotrophins did not appear to be localized to the same vesicles as goblet cell mucins.

CGCs also expressed all four NTs (green) as shown in Figure 3. The identity of cultured cells was determined using UEA-1 and appeared red. NGF, BDNF, and NT3 immunofluorescence was observed as punctate staining in the perinuclear area of the cultured cells. NT3 was also localized to the nucleus of the cultured goblet cells while NT4 was present in the cytosol of these cells (Fig. 3). There was some colocalization of the NTs and UEA-1 (yellow) indicating that goblet cell mucins and NTs could be packaged in the same secretory granules.

Expression of Neurotrophin Receptors Protein in Conjunctiva and CGCs

Because the four NTs were expressed in the conjunctiva and CGCs, we next determined whether the NTrs were also expressed. Membrane fractions from homogenates of conjunctival tissue, and CGCs were prepared. Homogenate samples from lacrimal gland and brain were used as positive controls. NTrs are heavily glycosylated and can appear as multiple bands by Western blot reflecting differential glycosylation that occurs during the normal trafficking of the Trk protein within cells.27 TrkA was detected in both CGCs and conjunctival tissue as well as in brain and lacrimal gland, at 140 kDa, corresponding to the full-length TrkA receptor protein (Fig. 4A). An additional band was observed in the CGCs at ~90 kDa. The additional band could be a result of the variable degree of glycosylation.

The NTr TrkB was detected as a band of ~80 kDa in the all tissues tested (Fig. 4B). This could correspond to the nonglycosylated receptor. In addition, several lower-molecular-weight bands were also present, with CGCs having numerous lower-molecular-weight proteins. These proteins could be truncated forms of TrkB.

TrkC was detected as major bands at approximately 140 and 100 kDa in the brain, conjunctiva, and CGCs (Fig. 4C). The lacrimal gland had a major band at approximately 100 kDa. It is known that TrkC can be present as truncated versions in cells.28 The 100-kDa band present in all tissues could be a truncated form.

The p75 receptor was present as a band at about of 75 kDa in brain, lacrimal gland, conjunctiva, and CGCs (Fig. 4D). Higher-molecular-weight bands were also visible in the brain and lacrimal gland.

Negative control experiments included the omission of the primary antibody. No bands were detected under this condition (data not shown).

Immunolocalization of Neurotrophin Receptors in Conjunctiva and CGCs

Immunofluorescence staining for NTrs in the conjunctiva is shown in Figure 5. Immunoreactivity to TrkA and appeared red. NGF, BDNF, and NT3 immunofluorescence was observed as punctate staining in the perinuclear area of the cultured cells. NT3 was also localized to the nucleus of the cultured goblet cells while NT4 was present in the cytosol of these cells (Fig. 3). There was some colocalization of the NTs and UEA-1 (yellow) indicating that goblet cell mucins and NTs could be packaged in the same secretory granules.

Immunofluorescence experiments performed with labeled antibodies directed against NGF, BDNF, NT3, and NT4 (green). The lectin UEA-1 conjugated to rhodamine was used to localize the secretory vesicles of goblet cells (red). Images are representative of cells cultured from three individual animals. Magnification ×200.
membranes of stratified squamous cells and basal lateral membranes of the goblet cells. p75 was also found on filamentous structures that penetrated the epithelium that could be nerves.

In CGCs, TrkA, TrkC, and p75 were located in cell nuclei, although the nuclear staining by TrkC and p75 was not homogeneous. In addition, TrkA and TrkB had a punctuate localization in the cytoplasm of the cells (Fig. 6).

Negative control experiments included the omission of the primary antibody. No immunoreactivity was detected in these experiments.

Effect of Neurotrophins on CGC Glycoconjugate Secretion

CGC glycoconjugate secretion in response to increasing concentrations (10^{-12} - 10^{-8} M) of NGF, BDNF, NT3, and NT4, and the cholinergic agonist carbachol at 10^{-4} M (the positive control) is shown in Figure 7. NGF stimulated goblet cell secretion in a concentration-dependent manner in CGCs (Fig. 7A). Glycoconjugate secretion was statistically significantly increased with 10^{-10} to 10^{-5} M NGF. Maximum secretion was stimulated by 10^{-9} M NGF, which increased secretion 1.9 ± 0.3-fold over basal. BDNF also stimulated secretion in a dose-dependent manner but only 10^{-9} M significantly induced glycoconjugate secretion compared with basal (1.7 ± 0.3-fold above basal, Fig. 7A). In these cells, carbachol, which is a known goblet cell agonist, increased CGC glycoconjugate secretion 1.9 ± 0.1-fold above basal. Neither NT4 nor NT3 stimulated goblet cell secretion (Fig. 7B). In these experiments, carbachol stimulated secretion 2.4 ± 0.5-fold over basal.

Effect of Neurotrophins on Goblet Cell Proliferation

As measured by the WST-8 assay, neither NGF, BDNF, NT3, nor NT4 at any concentration tested (10^{-12} - 10^{-6}) induced goblet cell proliferation after 24 hours of incubation (Fig. 8). In the same experiment, FBS (10%), the positive control increased CGC proliferation 1.9 ± 0.2- and 2.4 ± 0.5-fold above basal.

DISCUSSION

In the present study we examined the expression of NTs and NTrs in the conjunctiva and CGCs. Using Western blot analysis, immunofluorescence, and biochemical assays we demonstrated the presence and localization of NTs and NTrs in the conjunctiva and CGCs, and their ability to stimulate mucin secretion, but not cell proliferation. The conjunctival epithelium is therefore similar to the lacrimal gland, cornea, retina, brain, and lens in which multiple NTs and NTrs have been shown to be present.

NTs are synthesized as a pro molecule before enzymatic cleavage to the mature molecule. Both the pro form and the mature molecule can be biologically active. In addition to these two forms, NTs are present in many cell types as dimers and can be glycosylated. NTrs are heavily glycosylated and can be present in cells as truncated forms. This makes the identification of NTs and their receptors via Western blot analysis difficult due to the multiple bands detected. The conjunctiva is similar to other tissues, in which multiple bands for NTs and NTrs were detected via Western blot analysis.

NTs were distributed in the cytoplasm of cells throughout the entire conjunctival epithelium and stroma. In CGCs, NT4 was distributed throughout the goblet cell cytoplasm whereas NGF- and BDNF-containing vesicles were located in the perinuclear area. Unlike in conjunctival goblet cells, these vesicles appeared to colocalize with UEA-1 lectin staining in the secretory vesicles of CGCs. These results suggest that NTs form part of the secretary product in CGCs. It is possible that, in CGCs, the localization of the NTs is due to a loss of polarity of the cells in culture. In addition, it is interesting that NTs were found in lateral and basal membranes of conjunctival goblet cells. These observations are in contrast with other tissues in which NGF is secreted from secretory vesicles. Aside from neurons, many other cell types and tissues secrete neurotrophins in mouse submandibular gland, NGF is produced in high concentrations by the granular convoluted tubule cells and is secreted into the saliva.

In CGCs, NTrs are localized to the nucleus of these cells. In the conjunctiva, only TrkC was found to be predominantly located in cell nuclei of stratified epithelial cells and goblet cells. These differences in localization of the NTrs in the conjunctiva and CGCs could indicate differential trafficking of these receptors during protein synthesis. It is unclear why NTs are present in the nucleus of the cultured cells. It is known, however, that NTs are present on intracellular membranes such as the Golgi apparatus and can be recruited when activated by G-protein–coupled receptors. p75 can change its localization with stimulation with NGF.
Despite the presence of all members of the neurotrophin family of growth factors and their receptors in the conjunctival epithelium and in CGCs, only NGF and BDNF stimulated CGC glycoconjugate secretion. In addition, TrkA and TrkC, the appropriate receptors for NGF and BDNF, are present in CGCs. Thus, neurotrophins, in addition to cholinergic agonists and VIP, could regulate goblet cell secretion through an autocrine or paracrine mechanism. However, nothing is known about how NGF and BDNF are released or the signal transduction pathways used by NGF and BDNF to stimulate goblet cell glycoconjugate secretion. It has been shown that NTs, acting through the Trk receptors activate several signaling pathways including the Ras/Raf/MAPK pathway.40 We have shown that activation of MAPK by cholinergic agonists leads to glycoconjugate secretion.41 Thus, it is possible that NGF and BDNF also activate MAPK to stimulate goblet cell secretion.

It well established that the low-affinity NGF receptor p75 cooperates with TrkA to enhance its responsiveness to NGF. p75 was localized to the stratified squamous cells of the conjunctiva and in goblet cell membranes and thus could interact with TrkA, -B, or -C. In CGCs, all receptors were present in the nucleus where they could also interact. Neurotrophins could also be released from the conjunctival cells and interact with the corneal epithelial cells. NGF is increased in tears after corneal wounding in dogs and rat cornea shows increased ability to bind NGF after a corneal wound.22,42 In addition, NGF accelerates the rate of wound healing in humans.15 In addition, NGF induced the expression of p75 receptors in fibroblasts from human cornea that constitutively expressed TrkA. The effect was to increase the migration of fibroblasts, but not their proliferation or collagen production.44 Thus, NTs released from conjunctival cells could modulate corneal cell proliferation in response to injury.

The presence of NTs and NTrs in the conjunctiva may represent a key point in the reflex activation by the ocular surface nerves and autonomic nerves innervating the goblet cells. Neuromodulation of goblet cell mucin secretion. It is also possible that the presence of NTs in the conjunctiva serves as an alternative source of NTs for maintaining the survival and differentiated phenotype of the conjunctival epithelial cells including the goblet cells and the sensory parasympathetic and sympathetic neurons that innervate the conjunctival tissue.15 NTs present in the conjunctiva may also promote the neural innervation of the ocular surface. In the conjunctival epithelium, NTs could stimulate conjunctival goblet cell secretion as well as maintain the differentiation and survival of the "neural arc," made of afferent sensory and efferent parasympathetic and sympathetic nerves.

In conclusion, we showed that the NTs NGF, BDNF, NT3, and NT-4 and the NTrs TrkA, TrkB, TrkC, and p75 are expressed by the conjunctival tissue and CGCs. In addition, NGF and BDNF stimulate goblet cell mucin secretion. Thus, NTs and NTrs could play a role in the health and maintenance of the conjunctival and corneal epithelia.

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**References**

Neurotrophins in Rat Conjunctival Goblet Cells


