Patterned Expression of BDNF and NT-3 in the Retina and Anterior Segment of the Developing Mammalian Eye

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PURPOSE. The neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are hypothesized to play an important role in vertebrate eye development because of their patterned expression in the developing and adult neuroretina, their regulated response to retinal and optic nerve injury, and the effects of altered neurotrophin signaling on retinal development. To further characterize the role of these neurotrophins in mammalian eye development and maintenance, the pattern of expression of BDNF and NT-3 was analyzed in the developing and mature mouse eye.

METHODS. Using mouse strains in which the reporter gene lacZ, encoding the enzyme β-galactosidase, was targeted to either the BDNF or NT-3 locus, the expression of BDNF and NT-3 in the eyes of mice heterozygous for these mutations was analyzed by enzyme histochemistry during embryogenesis, postnatal development, and adulthood.

RESULTS. BDNF and NT-3 expression were first observed in the inner and outer segments of the developing optic cup at embryonic days 10.5 to 11.5. As the retina matured, BDNF expression was restricted to retinal ganglion cells and a subset of cells in the inner nuclear layer (INL), whereas NT-3 expression was confined to a small subset of cells in the INL and ganglion cell layer. Both neurotrophins were expressed within the developing retinal pigment epithelium. In the anterior segment, BDNF and NT-3 were expressed at high levels in the developing and mature ciliary epithelium. In the lens and cornea, however, these neurotrophins displayed distinct patterns of expression during development and adulthood. BDNF expression was found in the lens epithelium, immature trabecular meshwork, corneal endothelium, and corneal epithelium, whereas NT-3 expression was confined to the corneal epithelium.

CONCLUSIONS. BDNF and NT-3 exhibit different, yet overlapping, patterns of expression during the development and differentiation of the mouse eye. In addition to the neuroretina, the spatiotemporal expression of BDNF and NT-3 may play an important role in the development and maintenance of the lens, ciliary body, trabecular meshwork, and cornea. (Invest Ophthalmol Vis Sci. 1999; 40:2996–3005)

Neurotrophic factors are a class of cell signaling molecules critical for the differentiation and survival of neuronal cells. One family of neurotrophic factors, the neurotrophins, encompasses several proteins related to nerve growth factor that initiate signal transduction in responsive cells through the Trk family of receptor tyrosine kinases or the 75–kDa low-affinity neurotrophin receptor (p75NTR). Members of the neurotrophin family and their preferred Trk receptor include nerve growth factor (NGF)–trkA, brain-derived neurotrophic factor (BDNF)–trkB, neurotrophin-3 (NT-3)–trkC, and neurotrophin-4/5 (NT-4/5)–trkB.

As derivatives of the primitive vertebrate forebrain, the optic nerve, retina, ciliary body, and iris are ocular tissues whose differentiation and maintenance are conjectured to require neurotrophic support. For NGF, BDNF, and NT-3, this hypothesis has received preliminary support from several lines of experimentation: neurotrophin and Trk receptor localization studies3–11; neurotrophin support of retinal ganglion cell (RGC) and retinal pigment epithelium (RPE) tissue cultures6,12–14; the rescue of injured ocular tissue by exogenous neurotrophins15–20; and the effect of augmenting or inhibiting neurotrophin signaling on eye development.21–24 Although these studies suggest important roles for the neurotrophins in the differentiation and survival of certain vertebrate retinal cell populations, little is known about the functional role of neurotrophin signaling during mammalian eye development. In Xenopus and chicken, the developmental expression of BDNF and NT-3 has been examined in detail and has suggested potential roles for these neurotrophins in the autocrine and paracrine support of RGC and amacrine cell populations.5,6,25 In the mammalian eye, however, data on the developmental expression of neurotrophins remain quite limited.1 BDNF ex-
expression has been examined only during postnatal development, and NT-3 expression has yet to be described.

METHODS

Animals

All procedures involving animals were in accordance with the guidelines of the Institutional Animal Care and Use Committee (University of Colorado), the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23), and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The NT-3lacZneo mouse strain was constructed by gene targeting in embryonic stem (ES) cells as described in Farinas et al.26 The BDNFlacZneo mouse strain was constructed by homologous recombination in ES cells using standard techniques (Vigers et al., in preparation). In the targeting construct, the BDNF coding region was replaced, beginning at the initial methionine codon, with the Escherichia coli lacZ gene and the PGKneo selectable marker. In each mouse strain, transcription from the targeted genomic locus is predicted to produce active β-galactosidase (β-gal) protein rather than the neurotrophin. Heterozygous embryos, pups, and adults were identified by polymerase chain reaction analysis of tail tissue DNA using primers specific for lacZ and either NT-3 or BDNF 5′-genomic sequence.

Tissue Preparation

Eye tissue was harvested from embryos (embryonic days 10.5–18.5 [E10.5–E18.5]), pups, postnatal days 0 to 15 (P0–P15), and adult mice (P35). The stages sampled were selected to encompass the entire period of morphologic and cellular differentiation of the mouse eye.27,28 Whole embryos from E10.5 to E14.5 were fixed for 4 hours in fixation buffer (4% paraformaldehyde in 0.1 M sodium phosphate, pH 7.4). Eyes or whole heads were removed from E15.5 to E18.5 embryos and fixed in fixation buffer. Pups and adult animals were deeply anesthetized and perfused with fixation buffer. Then the eyes and optic nerves were removed and postfixed an additional 30 minutes in fixation buffer.

After fixation, eyes were washed twice for 10 minutes in solution C (0.1 M phosphate buffer, pH 7.4, 2 mM MgCl₂, 5 mM EGTA, 0.01% sodium deoxycholate, 0.02% Nonidet P40) and incubated overnight at 37°C with gentle agitation in staining solution C (0.1 M phosphate buffer, pH 7.4, 2 mM MgCl₂, 5 mM EGTA, 0.01% sodium deoxycholate, 0.02% Nonidet P40) and incubated overnight at 37°C with gentle agitation in staining buffer (solution C containing 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 1 mg/ml X-gal). Stained eyes were then washed several times in solution C, dehydrated through a graded ethanol series, cleared in xylene, and embedded in Paraplast X-tra (Sherwood Medical). Embedded tissue was sectioned at 7 μm and counterstained with neutral red after rehydration. Sections were photographed using a Nikon Optiphot microscope using neutral density filters and daylight print film. Artwork was arranged from color prints, digitally scanned, and the final output generated on a Kodak LED printer. No X-gal staining was observed in equivalently processed control tissues obtained from wild-type mice (data not shown).

RESULTS

The β-Gal Reporter System and Neurotrophin Expression

β-gal has proven to be an excellent reporter of gene expression in a variety of tissues across many different species. Because of the lack of background activity, β-gal provides an excellent surrogate marker for monitoring neurotrophin expression in the eye. Nevertheless, the turnover, intracellular localization, transport, and perdurability of this enzyme are likely to be distinct from those of its neurotrophin counterparts. In addition, insertion of the lacZ gene into the neurotrophin locus may result in unforeseen transcriptional leakiness in a variety of ocular tissues. For these reasons, β-gal expression may not exactly correlate with that of the endogenous BDNF or NT-3 protein.

Despite these caveats, through the remainder of this manuscript “β-gal expression” will be reported as either “BDNF expression” or “NT-3 expression,” depending on the transgenic strain being examined. This approach will be taken for two reasons. First, it will avoid excessive complexity in the language. And second, β-gal expression in the central nervous system and neuroretina of the respective reporter strains has generally agreed quite well with prior in situ hybridization and immunohistochemical studies of murine BDNF and NT-3 expression (Vigers et al., in preparation).

BDNF Expression in Developing and Mature Retinas

BDNF expression in the developing neuroretina could be detected in the developing pigment epithelium at approximately E11.5 (Fig. 2B). At E12.5, expression was observed along the inner margin of the peripheral sensory retina and throughout the outer pigment epithelium (Fig. 1A). Initial BDNF expression in the developing retina at this stage was often asymmetrical, but there was no reproducible nasal or temporal bias to the asymmetry. By E14.5, BDNF expression was observed as a confluent band encompassing the anterior and vitreal margins of the optic cup (Fig. 1B). At this stage, the vitreal margin of the optic cup is populated by nonreplicating cells destined to reside in the ganglion cell layer (GCL),29 whereas the anterior margins are populated by cells that will eventually differentiate to form the ciliary body and iris. At E14.5, BDNF continued to be produced by the underlying retinal pigment epithelium (RPE); however, resolution of the X-gal product was diminished by the increasing level of pigment deposition (Fig. 1B). Outside the developing retina, BDNF expression was detected in cells of the primitive hyaloid plexus (Fig. 1B).

By E17.5, the sensory neuroretina becomes demarcated into distinct inner and outer neuroblastic layers.28 At this stage, the differentiating cells in the inner neuroblastic layer expressed BDNF robustly (Fig. 1C). By birth, a more mature two layer retina was apparent, with BDNF expression restricted to the GCL and distinct cells along the inner margin of the outer nuclear layer (Fig. 1D). In this tissue section, BDNF expression was observed in the underlying RPE that was artificially separated from the overlying sensory neuroretina. As the retina matured, BDNF production remained essentially unchanged, with expression restricted to the GCL and sporadic cells within
the inner margin of the inner nuclear layer (INL; Fig. 4B). No spatial bias to BDNF expression was observed in wholemount tissue from adult retinas (data not shown). A summary of BDNF expression in the developing and mature retina is presented in Table 1.

**BDNF Expression in Developing and Mature Lenses**

BDNF expression could be detected in the developing lens at E10.5, before any observable expression in the underlying tissue from adult retinas (data not shown). A summary of BDNF expression in the developing and mature retina is presented in Table 1.

**FIGURE 2.** Distribution of BDNF in the developing eye of BDNF-lacZ heterozygous mice. (A) BDNF (E12.5; magnification, ×200) is expressed in the primitive lens epithelium and along the inner margin of the developing optic cup (arrowhead, optic cup; double arrowhead, lens vesicle). Additional X-gal product is evident in the RPE (open arrow). (B) BDNF (E14.5; magnification, ×100) expression increases along the inner and anterior margins of the optic cup and within the RPE (open arrow). (C) BDNF (E17.5; magnification, ×400) is expressed by the majority of cells in the inner neuroblastic layer (arrow). (D) BDNF (P0; magnification, ×400) is expressed by maturing RGCs and by cells in the RPE (arrow). (E) BDNF (P15; magnification, ×200) is expressed in the lens epithelium (arrow), ciliary body (cb), and ciliary muscle (arrowhead). Diffuse X-gal product is present within the reticular trabecular meshwork adjacent to the ciliary muscle. (F) BDNF (P15; magnification, ×200) is expressed by the corneal endothelium and epithelium (arrow, lens epithelium; arrowhead, corneal epithelium; double arrowhead, corneal endothelium).
optic cup (Fig. 2A). Expression at this stage was limited to the primordial lens epithelium and diminished as the epithelial cells invaginated to form the lens pit. Additional sporadic expression was observed in the periciliar mesenchyme (Fig. 2A). By E12.5, primary lens fibers had begun to elongate and almost completely filled the lens vesicle. At this stage, BDNF expression was limited to the most anterior portion of the lens epithelium (Fig. 1A). At E14.5, a larger portion of the lens epithelium expressed BDNF; however, expression remained conspicuously absent from the equatorial transitional zone (Fig. 1B). As development proceeded, BDNF expression remained restricted to the lens epithelium (Figs. 1B, 1E, 1F, 4D). At all stages, the intensity of β-gal staining remained highest in the germinative zone (Fig. 1E, Fig. 4D, and data not shown).

**BDNF Expression in the Ciliary Body, Trabecular Meshwork, and Cornea**

BDNF expression becomes accentuated in the rim of the developing optic cup at E14.5 (Fig. 1B). As development proceeded, BDNF expression intensified, and by E17.5, a morphologically distinct primitive ciliary epithelium was apparent (data not shown). At P15, the mature morphology of the ciliary body and iris were evident, and BDNF expression could be observed quite readily in the ciliary epithelium, the iris epithelium, and the longitudinal ciliary muscles (Fig. 1E). BDNF expression continued in these anterior segment tissues through adulthood (Fig. 4D). Because of the abundance of pigment in animals older than E17.5, the level of expression of BDNF in the underlying pigmented ciliary body and iris remains in question.

Expression of BDNF in the developing cornea could be detected first at E14.5 (Fig. 1B). At this stage, migrating neural crest cells have just condensed along the inner surface of the developing cornea to form a morphologically distinct corneal endothelium (as shown in Fig. 1B). As development proceeded, BDNF continued to be expressed uniformly by the cells of the corneal endothelium; sporadic expression was observed within the overlying multilayered corneal epithelium (Fig. 1F). During adulthood, expression continued in both layers of the cornea, albeit at less intense levels in the corneal endothelium (as shown in Fig. 4F). BDNF expression was not observed in the adjacent conjunctiva (Fig. 4D and data not shown).

BDNF-expressing cells could be observed first in the future angle of the anterior chamber at E14.5 (Fig. 1B). At E17.5, a diffuse reticular network of cells had collected in the angle of the expanding anterior chamber (as shown in Fig. 2C). These spindle-shaped cells were morphologically similar to nearby BDNF-expressing cells that have formed the adjacent corneal endothelium. At P0, BDNF expression continued within a trabecular meshwork that had coalesced and acquired a more mature morphology (as shown in Fig. 2D). By P15, BDNF expression was diminished, although still evident within the mature cells of the chamber angle (as shown in Fig. 1E). By adulthood, however, X-gal product was no longer evident in the trabecular meshwork and appeared restricted to the adjacent ciliary muscle (as shown in Fig. 4D).

**NT-3 Expression in Developing and Mature Retinas**

NT-3 expression could be detected first in the outer layer of the developing optic cup at approximately E10.5 (data not shown). By E11.5, NT-3 expression could be observed in the developing pigment epithelium and cells in the mid-periphery of the inner sensory neuroretina (Fig. 3A). As pigmentation within the RPE
increased, β-gal staining became increasingly obscured; however, expression could be observed as late as P0 (Fig. 3F). NT-3 expression in the developing optic cup was complemented by additional neurotrophin production in the region of the optic stalk and future optic nerve (Figs. 3A and 3E). By E13.5 to 14.5, the cells expressing NT-3 within the inner retinal layer were significantly diminished, and a new focus of NT-3 expression became evident at the periphery of the optic cup coincident with the location of the future anterior uvea (as shown in Fig. 3B). By E16.5, retinal NT-3 expression was confined to the anterior margin of the optic cup despite the lack of any clear morphologic evidence of a definitive ciliary body and iris (Figs. 3C and 3D). At this stage of development, NT-3 expression was notably absent from the sensory retina.

At E17.5, NT-3 expression reappeared in the sensory retina in cells at the inner boundary of the outer neuroblastic layer (Fig. 3E). Cells within this portion of the neuroretina are actively replicating, as determined by their uptake of radioactive thymidine.29 A large majority of these cells differentiate and migrate to their final destinations in the inner portion of the INL and the photoreceptor layer, whereas a sporadic few eventually reside in the GCL.29 Consistent with this observation, at birth, NT-3 expression was evident along the inner edge of the outer nuclear layer and sporadically within the GCL (Fig. 3F and data not shown). As the retina matured, the pattern of NT-3 expression remained stable. β-gal staining was limited to cells within the inner portion of the INL and the GCL (Fig. 4A). A summary of the pattern of NT-3 expression in the neuroretina is presented in Table 1.

**NT-3 Expression in the Developing Ciliary Body and Cornea**

As mentioned previously, NT-3 expression could be detected first in the presumptive ciliary body and iris at E14.5 (Fig. 3B). Similar to that observed for BDNF, NT-3 expression in the maturing ciliary epithelium occurred before the establishment of any mature morphologic characteristics (Figs. 3C and 3D). The ciliary epithelium continued to express NT-3 through adulthood (Fig. 4B). Contrary to that observed for BDNF, NT-3 expression was never observed in the ciliary muscle, trabecular meshwork, or lens.

NT-3 expression could be observed early in development in the primitive epithelium overlying the developing optic cup and lens vesicle (Fig. 3A). By E14.5, as a more definitive cornea began to take shape, NT-3 expression was observed diffusely in the corneal epithelium and sporadically in the underlying mesenchymal stroma (Fig. 3B). As the early anterior chamber expanded, NT-3 expression remained restricted to the corneal epithelium; additional strong expression was observed in the epithelium of the overlying eyelid margins (Figs. 3C and 3D). In the adult mouse, NT-3 was found diffusely within the corneal epithelium and was absent from the corneal endothelium and adjacent conjunctiva (as shown in Figs. 4C and 4E).
DISCUSSION

Potential Roles for BDNF and NT-3 in the Retina and RPE

In the mouse retina, the timing and pattern of BDNF and NT-3 expression are generally consistent with that previously reported in other vertebrate species (Table 1).5-7,30 BDNF expression could be detected early in retinal development at E12.5 (Fig. 1A) and became rather prominent along the vitreal margins of the optic cup by E14.5 (Fig. 1B), approximately the time at which the first RGCs are maturing.27,29 Consistent with a potential autocrine role in RGC support, BDNF expression remained robust within the maturing GCL during eye development (Figs. 1C and 1D), and by adulthood BDNF was generally confined to the GCL, with only sporadic cells along the inner margin of the INL expressing the neurotrophin (Fig. 4B). The abundance and distribution of the BDNF-expressing cells in the INL were consistent with their being either a subset of amacrine cells or displaced RGCs.31,32 A similar pattern of BDNF expression in the neural retina has been observed in the frog5 and rat,7 suggesting that the function of this neurotrophin in vertebrate eye development has remained relatively conserved.

Multiple lines of evidence suggest that BDNF function may involve autocrine or paracrine trophic support of RGCs. First, trkB receptors are expressed in RGCs in multiple species.4,6,9,10 Second, BDNF promotes survival of RGC cultures and nerve fiber outgrowth from primary retinal explants.5,6,33 And, third, BDNF ameliorates RGC death after traumatic optic nerve injury.15,17,34 Interestingly, optic nerves from bdnf−/− mice are reduced in size and hypomyelinated, suggesting that BDNF may be important for RGC maturation rather than survival.51 Recent studies by Herzog and von Bartheld25 in chickens have indicated that the majority of BDNF in RGC neurons is derived from amacrine and bipolar cells in the INL. Our results in the mouse with the b-gal reporter system, however, are not consistent with this notion. Although the disparity between the two findings may be a result of the potential drawbacks of the reporter system (see the Results section), two lines of evidence argue that the disparity may represent a fundamental distinction in BDNF signaling between the two species. First, in the BDNFlacZneo strain, b-gal expression is temporally and spatially coincident with the development of RGCs.27,29 Consistent with a potential autocrine role in RGC support, BDNF expression remained robust within the maturing GCL during eye development (Figs. 1C and 1D), and by adulthood BDNF was generally confined to the GCL, with only sporadic cells along the inner margin of the INL expressing the neurotrophin (Fig. 4B). The abundance and distribution of the BDNF-expressing cells in the INL were consistent with their being either a subset of amacrine cells or displaced RGCs.31,32 A similar pattern of BDNF expression in the neural retina has been observed in the frog5 and rat,7 suggesting that the function of this neurotrophin in vertebrate eye development has remained relatively conserved.

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In addition to its potential role in autocrine or paracrine RGC signaling, BDNF produced by RGCs may provide trophic support for adjacent amacrine cells in the INL or neurons in the

FIGURE 4. Distribution of NT-3 and BDNF in the adult mouse eye. (A, C, E) NT-3-lacZ heterozygous mice; (B, D, F) BDNF-lacZ heterozygous mice. (A, B) NT-3 and BDNF are expressed in the mature retina (IPL, inner plexiform layer; OPL, outer plexiform layer; PRL, photoreceptor layer; magnification, ×400). (C) NT-3 is abundant in the ciliary epithelium and cornea (arrow, ciliary body; double arrow, corneal epithelium; magnification, ×200). (D) BDNF is expressed in the lens, ciliary epithelium, and ciliary muscle (arrow, lens epithelium; cb, ciliary body; double arrow, ciliary muscle; magnification, ×200). No stain is evident in the adjacent trabecular meshwork. (E) NT-3 is specifically expressed within the corneal epithelium (arrow, epithelium; asterisk, endothelium; magnification, ×400). (F) BDNF is expressed in the adult corneal endothelium and epithelium (arrow, endothelium; arrowhead, epithelium; star, lens epithelium; magnification, ×200).
superior colliculus through retrograde and anterograde transport. Support for these potential signaling pathways comes from a variety of experimental systems. In the vertebrate retina, trkB immunoreactivity is localized to a subset of amacrine cells in the INL. Colocalization of trkB immunoreactivity and tyrosine hydroxylase immunoreactivity is observed in several species, suggesting that neurotrophin signaling may be important for the development and maintenance of the retinal dopaminergic pathway. In recent studies, modulation of BDNF and NT-3 signaling produced significant alterations in the level of tyrosine hydroxylase immunoreactivity, D2 dopamine receptor immunoreactivity, and dopaminergic cell morphology in the INL. In addition to local signaling, tectal neurons in the superior colliculus may derive support from anterograde transport of BDNF by RGCs. In chickens, anterograde neurotrophin transport from the retina to the optic tectum has been observed after intraocular administration of labeled BDNF, and in the mouse, striatal neurons have been shown to receive BDNF support primarily by anterograde transport from afferent projections.

In certain tissues and organisms, neurotrophin signaling may act negatively on cell survival and synapse formation both in vitro and in vivo. At various developmental stages, NGF, BDNF, and NT-4/5 have been shown to induce growth cone collapse in developing embryonic trigeminal sensory neurons, and trkA and p75 receptors have been shown to mediate apoptosis in susceptible oligodendrocyte and neuronal cell populations. BDNF may play a similar role in retinal development by modifying neuronal cell number or synapse formation. During postnatal eye development, RGCs are reduced in number through programmed cell death, and BDNF may play an active role in this process by signaling through p75 or variant trkB receptors. Recently, Rohrer and colleagues have shown an absence of electoretinographic responses in mice lacking trkB receptors. This electrophysiological abnormality may be the product of a delay in retinal maturation or an abnormality in photoreceptor synapse formation.

NT-3 expression in the developing mouse retina occurs in two distinct phases. Expression could be observed first at E10.5 to E11.5 in the outer epithelium of the developing optic cup. Soon afterward, NT-3 expression extended to the peripheral inner margin of the developing optic cup (Fig. 3A) and was rapidly restricted to the anterior margins by E14.5 (Fig. 3B). Whether the initial cells expressing NT-3 in the developing retina discontinue neurotrophin production, actively migrate to the peripheral rim, or are passively relocated to the periphery as the optic cup expands remains to be determined. At E17.5, NT-3 expression returned to the neuroretina along the inner margin of the outer neuroblastic layer (Fig. 3E). As mentioned previously, these cells are actively replicating, and a large majority differentiate and migrate to their final destinations in the INL. Consistent with this observation, NT-3 expression in postnatal and adult retinas is restricted to the inner margin of the INL and a scattered few cells in the GCL (Figs. 3F and 4A). A similar restriction of NT-3 expression to the INL has been observed in the developing chick eye, where NT-3 localization to the outer margin of the INL by in situ hybridization has suggested expression by inner plexiform cells and bipolar cells. In the mouse eye, however, NT-3 is produced in a portion of the INL where amacrine cells are located, and the pattern and timing of NT-3 expression from E17.5 through adulthood are consistent with this notion. The different INL localization of NT-3 in the mouse and chicken may represent a fundamental distinction in retinal neurotrophin signaling between the two species, or it may be a result of error in localizing the autoradiographic signal. Colocalization of neurotrophin expression with retinal cell–type specific markers in each species should help to resolve this issue.

Because of the absence of information regarding the localization of trkC receptors in the rodent retina, the role of NT-3 signaling in retinal differentiation and maintenance remains speculative. A prior study was unable to identify trkC immunoreactivity in the rat retina, and complementary in situ hybridization studies have not been reported. In the chicken, trkC mRNA is localized throughout the neuroretina and does not appear to correspond in any ordered fashion with NT-3 expression. NT-3 may have some role in the trophic maintenance of RGCs, given its ability to support Thy-1+ RGCs in culture and to prevent axotomy-induced RGC death. In support of this notion, grafting of anti-NT-3 hybridoma cells to developing chick embryos in ovo results in a reduced quantity of RGCs. The early NT-3 expression observed in the region of the future optic nerve might provide initial trophic support for RGCs as they extend their axons toward the optic chiasm (Figs. 3A and 3E). Alternative roles for NT-3 function in the developing eye include autocrine and paracrine support of amacrine cells within the INL.

Both BDNF and NT-3 are expressed by the developing RPE (Figs. 1, 2, and 3). Unfortunately, in the reporter strains, β-gal expression in the RPE becomes increasingly difficult to identify as development proceeds, because the accumulating cellular pigment obscures the X-gal product. The observed expression of BDNF and NT-3 in the developing RPE is consistent with prior studies in Xenopus, chicken, human, and bovine cells. Cultured human and bovine RPE cells demonstrate saturable binding of BDNF, NT-3, and NT-4/5; and BDNF promotes differentiation of human and bovine RPE cells in culture. Liu et al. have shown that in Xenopus BDNF plays an autocrine role in RPE differentiation and survival. However, because the RPE provides maintenance and support for the overlying photoreceptor cells, BDNF may indirectly support retinal cell survival and injury response. Indeed, in the rat retina, administration of exogenous BDNF or NT-3 significantly protects photoreceptor cells from light exposure damage. In RPE cultures, BDNF upregulates expression of basic fibroblast growth factor (bFGF), another growth factor that reduces light-induced photoreceptor injury. Because of the role of the RPE in absorbing stray light, regenerating visual pigments, and transporting active metabolites, it is possible that the ameliorating effects of BDNF and NT-3 administration on light-induced retinal injury are mediated through trophic action on RPE cells rather than through direct action on photoreceptor cells.

The Role of BDNF in Lens Development

BDNF is expressed by the primordial lens epithelium early in development and continues to be expressed by the lens epithelium through maturity in both the anterior and germinative zones (Figs. 1B, 1E, and 4D). A potential role for BDNF in lens development is intriguing and would represent the first example of neurotrophin support for nonneuronal tissue. Lens development has been shown to be dependent on the presence of a variety of growth factors and cytokines: acidic and basic.
Potential Roles for BDNF and NT-3 in the Ciliary Body, Developing Trabecular Meshwork, and Cornea

BDNF and NT-3 are expressed continuously by the ciliary epithelium through development and maturation (Figs. 1, 3, and 4). NT-3 expression by the ciliary epithelium has been previously noted in the adult mouse,5,39; however, BDNF expression in this tissue has not been previously reported. Neurotrophin production by the ciliary and iris epithelia may be important for autocrine support, for paracrine support of the underlying neural crest–derived stroma, or for support of distal ocular structures through secretion into the aqueous humor. Some role in autocrine support is suggested by the early expression of both these neurotrophins along the peripheral rim of the developing optic cup, well before the emergence of any uveal structures (Figs. 1B, 3B, and 3C). Ciliary epithelial secretion of neurotrophin, particularly BDNF, may be important for the trophic support of adjacent ocular tissues such as the lens and trabecular meshwork. Secretion of BDNF by the ciliary epithelium and primitive hyaloid plexus may directly support lens epithelial cells or participate in the induction of lens polarity by establishing a neurotrophin gradient across the aqueous and vitreous humors.39,55

In the developing anterior segment, BDNF expression is prominent in several tissues of neural crest origin: ciliary muscle, trabecular meshwork, and corneal endothelium. Given the importance of BDNF for the differentiation and support of neuronal cell populations derived from neural crest,56,57 BDNF may be analogously important in the development and maintenance of these nonneuronal neural crest–derived tissues. Similar to BDNF transported from skeletal muscle to spinal motoneurons,58 BDNF produced by ciliary muscle may provide trophic support to innervating axons. If so, then treatment with neurotrophins might be expected to ameliorate the pupillary denervation associated with injury to cells in the ciliary ganglion. Similar trophic effects in trabecular meshwork tissue and corneal endothelium may have important implications for treating glaucoma and corneal endothelial dystrophies and injuries.

Both NT-3 and BDNF are expressed by keratocytes in the corneal epithelium (Figs. 1, 3, and 4). Mechanistically, neurotrophin expression by the corneal epithelium may be important, not only for autocrine support but also for the trophic support of adjacent trigeminal nerve endings. NGF, NT-3, and BDNF have been shown to support developing trigeminal sensory neurons in vitro and in vivo.26,59–64 The most robust effects are observed after overexpression of NGF or deletion of its receptor trkA. Trigeminal neurons are increased by up to 117% in mice overexpressing NGF in the epidermis and are reduced by 70% to 90% in trkA-deficient mice.59,64 Similarly, corneal innervation and blinking response are drastically reduced in trkA-deficient mice.65 Interestingly, recent clinical studies have revealed a benefit to treating neurotrophic corneal ulcers with topical NGF.20 Similar modulation of BDNF or NT-3 levels may prove important for the treatment of corneal wounds, neuroparalytic keratitis, or cell proliferation after glaucoma filtration surgery.

The expression of BDNF and NT-3 in the anterior segment of the eye has important implications regarding the interpretation of experiments involving the intracocular transplantation of brain tissue. The elevated levels of BDNF and NT-3 in the anterior segment may contribute to the case with which transplanted neuronal tissue is grafted into the anterior chamber. Many of the brain tissues studied in this model, the cerebral cortex, hippocampus, cerebellum, cochlea, mesencephalon, and striatum,66–69 are very sensitive to the local concentration of BDNF and NT-3; and, as a result, intracocular double grafts may be artificially influenced by the high expression of these neurotrophins in adjacent anterior segment tissues. Nevertheless, the success of these transplantation experiments raises hope that nerve explants will represent a viable avenue for treating certain ocular injuries in the future.

In conclusion, BDNF and NT-3 are expressed in distinct overlapping patterns in developing and mature mammalian eyes. In the retina, BDNF is expressed by developing and mature RGCs, whereas NT-3 is expressed by a subset of cells in the INL; both neurotrophins are expressed by the RPE. In the anterior segment, neuronal and nonneuronal cells express BDNF and NT-3. NT-3 is expressed by the ciliary epithelium and corneal epithelium, whereas BDNF is produced by the lens epithelium, ciliary epithelium, ciliary muscle, developing trabecular meshwork, and corneal epithelium and endothelium. The novel finding of neurotrophin expression by structures within the anterior segment of the eye may have significant implications for the potential treatment of diseases of the anterior segment and retina.

Acknowledgments

The authors thank Frank Bozyan and Zachary Baquet for their expert assistance with tissue preparation and histology, Alison Vigers for sharing results before publication, and Steven Galetta for his generous assistance with the illustrations.

References


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