Neurotrophins and Their Receptors in the Tench Retina During Optic Nerve Regeneration

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ABSTRACT
To understand the role of neurotrophins in the visual system, we investigated the distribution of both neurotrophins and their receptors within the retina of a fish that has the capacity to spontaneously regenerate its optic nerve axons after lesion. Intact retinas and retinas from tench, whose optic nerve had been crushed, were analyzed by immunohistochemistry and in situ hybridization. Trk receptors were mainly immunolocalized in cells of the inner nuclear and ganglion cell layers, a distribution coincident with that of their mRNAs. Nerve growth factor (NGF) immunoreactivity was detected exclusively in Müller cell processes, and brain-derived neurotrophic factor (BDNF) was found in both neuronal bodies and Müller cell processes. Neurotrophin-3 (NT-3) was detected in most of the cell nuclei, and neurotrophin-4/5 (NT-4/5) was localized in fibers and in a few cells in the inner retina. An increase in both TrkA protein and mRNA was detected during axonal regeneration within the retinal ganglion cell layer, reaching a maximum 30 days postcrush and returning to normal levels by day 90, when optic nerve regeneration is almost completed in this fish. None of the other neurotrophins and receptors showed appreciable changes. The heterogeneous distribution patterns of neurotrophins and their receptors in fish retina, their differences from the distribution observed in other species, and the TrkA changes after optic nerve crush suggest an important role for these molecules in the normal physiology of the fish retina and during the regeneration process. J. Comp. Neurol. 404:321–331, 1999.

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Neurotrophins and their receptors are involved in neuronal survival, differentiation, and development (Levi-Montalcini, 1987; Barde, 1989; Korsching, 1993; Jelksa and Aguayo, 1994; Davies and Wright, 1995). The nerve growth factor (NGF) family of neurotrophins comprises NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4/5, NT-6, and zebrafish NT-7. Neurotrophins exert their biological activities by binding and activation of two types of transmembrane receptors: the Trk family of tyrosine protein kinases and the low-affinity receptor, p75NTR. The receptor for NT-6 has not been identified (Fig. 1).

According to the classical neurotrophic theory, neurons receive trophic support from their targets (Levi-Montalcini, 1987; von-Bartheld et al., 1996b). It is also currently accepted that neurotrophic influences can be mediated by anterograde (from afferent neurons), retrograde (from innervated neurons), and autocrine (responsive neurons themselves) support (Korsching, 1993; Curtis and Di Stefano, 1994; Linden, 1994; Davies and Wright, 1995;...
von-Bartheld et al., 1996b). Moreover, glial cells also have the capacity to respond to neurotrophins in an autocrine fashion (Jelks et al., 1993; Korschning, 1993).

**Neurotrophin receptors**

The patterns of distribution of neurotrophin receptors have been widely reported in the mammalian and avian retina but not in anamniote vertebrate retina. The first studies on TrkA and TrkB detected a restricted immunodistribution in the ganglion cell layer (GCL), Müller cells, and optic axons of rat retina (Jelks et al., 1993; Zanolato et al., 1993). Sandell et al. (1994) described Trk immunoreactivity in zebrafish retina, without distinguishing between the different Trk receptors. Rickman and Brecha (1995) immunolocalized both TrkA and TrkB in all retinal layers, except the photoreceptor layer (PRL) and optic nerve. Recently, these studies have been extended to other species, thus showing immunohistochemical label for TrkB, TrkC, and p75 NGF in mammals (Ernfors et al., 1992; Jelsma et al., 1993). Moreover, the distribution of all high- and low-affinity neurotrophin receptors, with specific antibodies against intracellular and/or extracellular domains, has been studied in detail in rat retina (Vecino et al., 1998b).

Immunoprecipitation studies have detected TrkB and TrkC receptors in the ferret retina (Allendoerfer et al., 1994) and binding experiments have shown the existence of TrkC in chick retina (Rodríguez-Tebar et al., 1993). Martin et al. (1995) showed the existence of five trk genes expressed in zebrafish. Expression of mRNAs for trkA (Ernfors et al., 1992), trkB (Ernfors et al., 1992; Jelks et al., 1993; Perez and Caminos, 1995), and trkC (Ernfors et al., 1992) has been detected in the neuroblast layer, in the inner nuclear layer (INL), and in the GCL of embryo, newborn, and adult rat retinas. In chick retina, trkC mRNA has been detected in all nuclear layers but mainly in the GCL and INL (Bovolenta et al., 1996; Hallböök et al., 1996). The p75NGF immunoreactivity in mammals has always been found in Müller cells and the INL, GCL and retinal pigment epithelium (RPE; Schattenman et al., 1988; Henderson, 1991; Vecino et al., 1998a,b).

**Neurotrophins**

Although expression of neurotrophins in the visual system has been extensively investigated, little has been reported on the immunolocalization of neurotrophins in the retina. Only recently, a detailed study on the distribution of the neurotrophins has been performed in adult rat retina (Vecino et al., 1998b). Moreover, immunohistochemical analysis of BDNF and NT-3 in the developing chick retina has demonstrated that labeling for these molecules predominates in the inner retinal layers (Das et al., 1997). By in situ hybridization analyses, NGF, BDNF, and NT-3 mRNAs have been detected in the avian retina during embryonic and adult development (Hallböök et al., 1996), and BDNF mRNA has been found in rat retina during postnatal development (Perez and Caminos, 1995). Neurotrophin mRNAs have not been detected in embryonic rat retinas (Ernfors et al., 1992). By Northern analyses, expression of NGF, BDNF, and NT-3 has been detected in chick, rat, and Xenopus retinas (Ebendal and Persson, 1988; Maisonnier et al., 1990; Cohen-Cory and Fraser, 1994).

The axons of the retinal ganglion cells of the fish are able to regenerate after optic nerve damage and reestablish their appropriate pattern of synaptic connections in the optic nerve, thus recovering visual function. Regeneration of the fish retinal ganglion is enhanced by NGF (Turner et al., 1981; Yip and Grafstein, 1982). This is a unique property of the visual system of the fish and has not been observed in mammals (Sperry, 1948; Murray, 1976; Johns, 1977; Meyer, 1978; Grafstein, 1986). The fish used in the present and previous studies is the tench (Tinca tinca L., 1758) whose retina continues to grow throughout life from a small proliferative marginal area and whose optic nerves can regenerate after crushing (Vecino et al., 1998c).

The aim of the present study was to investigate the distribution of neurotrophins and their receptors within the retina of the tench and to compare the results found with the distribution of these molecules in mammalian retinas. We studied the cellular localization of all neurotrophins and their receptors in intact tench retinas and in retinas at different stages during the regeneration of ganglion cell axons following optic nerve crush. For this study, we used immunohistochemical and in situ hybridization techniques.

**MATERIALS AND METHODS**

**Animals**

Adult tench 14-16 cm in length were obtained from a commercial supplier. Experiments were carried out in accordance with the European Union guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Following anesthesia with 0.03% MS-222 (methanesulfonate salt; Sigma, St. Louis, MO), fish were transcardially perfused with 0.6% saline solution and 4% paraformaldehyde (PF), 15% picric acid in 0.1 M phosphate buffer (pH 7.3; PB). The eyes and optic nerves were dissected out. A small cut was made in the cornea of each eye to improve fixation of the retina. The tissues were postfixed overnight at 4°C in the same fixative, washed in PB, transferred into PB containing 25% sucrose, and embedded in Tissue Tek (Leika). Cryostat sections (12–16 μm thick) of the retina and the optic nerves were made, placed on gelatin-coated slides, and processed for immunohistochemistry.

For optic nerve crush, fish were deeply anesthetized, and the left optic nerve was crushed extraorbitally with forceps, taking care to leave the optic artery intact. One, 4, 7, 15, 20, 30, 90, 120, 210, and 250 days following optic nerve crush, the animals were anesthetized and treated as
described above. At least three animals were used for each survival period to study the distribution of NGF, BDNF, NT-3, NT-4, TrkA, TrkB, TrkC, and p75 immunohistochemically and the trkA, trkB, and trkC mRNAs by in situ hybridization.

Antibodies

In the present study, the following polyclonal anti-peptide antibodies against neurotrophin receptors were used: anti-350, an antigenic peptide corresponding to the extracellular amino acids 267–285 of rat TrkA; anti-TrkB antibodies against the extracellular (amino acids 76–96) and intracellular (amino acids 463–502, at the juxtamembrane region) domains of rat TrkB (Allendoerfer et al., 1994); anti-Y15E, an anti-peptide antibody raised against a conserved sequence corresponding to amino acid residues 639–652 of kinase domain of chicken TrkC (Becker, 1997); and anti-p75, a polyclonal antiserum raised against the cytoplasmic domain of the human low-affinity neurotrophin receptor (Chao et al., 1996). The antibodies used against the neurotrophins were monoclonal anti-NGF antibody (anti-mouse-b-2.5S; Boehringer Mannheim, Germany); polyclonal anti-BDNF antiserum directed to amino acids 168–177 of human BDNF (Patterson et al., 1996); polyclonal anti-NT-3 antibody, made against a peptide corresponding to amino acids 178–186 of human NT-3 (Dr. D. Kaplan, unpublished observations); and polyclonal anti-NT-4/5 antiserum raised against a peptide corresponding to amino acid residues 42–52 of rat NT-4/5 (Funakoshi et al., 1995).

Immunohistochemical procedure

Sections were rinsed in phosphate buffered saline solution, pH 7.3, containing 0.25% Triton X-100 (PBST), and immersed in 3% hydrogen peroxide in absolute methanol for 10 minutes, thus blocking the endogenous peroxidase activity. After washing in PBST, tissues were preincubated for 1 hour at room temperature (RT) with blocking solution containing PBST and 1% bovine serum albumin (BSA; Fraction V, Sigma). Sections were then incubated in primary antibodies, diluted to 1:1,000–3,000 (except anti-NGF antibody, which was used at 1:10) in PBST-BSA, for 12–18 hours at RT in a humid chamber and then washed in PBST and incubated for 1 hour at RT with 1:200 biotinylated secondary antibody (anti-rabbit or antimouse antibodies; Vector Laboratories, Burlingame, CA).

After washing with PBST, sections were incubated with avidin-biotin-peroxidase complex (ABC) reagent (Vector) for 1 hour, rinsed in PBST and Tris-HCl buffer (pH 7.6), and incubated with 0.05% diaminobenzidine and 0.03% H2O2 in 0.1 M Tris-HCl. All buffer washes were made with constant agitation. The tissues were washed in phosphate buffered saline (PBS), dehydrated, mounted, and examined under light microscopy.

Labeling specificity was assessed by (1) omission of the primary antiserum by replacing it with PBST-BSA, (2) omission of anti-rabbit antibody, (3) omission of ABC reagent, and (4) primary antibodies because some receptors were preadsorbed with their respective antigenic peptides (2–10 µg/ml of serum).

In situ hybridization

Cellular mRNAs for trkA, trkB, and trkC were detected with 35S-labeled (New England Nuclear, Boston, MA) or digoxigenin- (DIG; Boehringer Mannheim) labeled RNA probes generated by transcribing linearized plasmids containing cDNA clones of the desired gene by using appropriate RNA polymerases. Probes used were mouse trkA, pJA2 containing a 1,086-bp cDNA insert corresponding to nucleotides 1372–2457 of human trkA (Martín-Zanca et al., 1989); mouse trkB, pFRK16 containing a 483-bp insert corresponding to nucleotides 1181–1663 of mouse trkB (Klein et al., 1990); chicken trkB, pMTN-3 containing a 695-bp insert corresponding to nucleotides 1784–2493 of full-length avian trkB (Garner and Large, 1994). Retinas from intact tench and from tench living 1, 7, and 30 days after optic nerve crush were immersed in 4% PF for 2 hours and rinsed in 0.1 M PBS, immersed in 25% sucrose, and cut on a cryostat (12–16 µm). Sections were treated with 4% PF, 0.2 M HCl, and 0.1 M triethanolamine-0.25% acetic anhydride, dehydrated, and air dried. The sections were then preincubated for 1 hour with hybridization buffer (HB): 50% deionized formamide; 1× Grundmix (1 M Tris-HCl, 0.25× Denhardt’s solution, 50 µg/ml yeast tRNA, 25 mg/ml fish DNA, 25 µM EDTA); and 250 mM NaCl, 0.1 M dithiothreitol (only for isotopic procedure), and 8% dextran sulphate. For hybridization, sections were incubated at 42°C for 16–18 hours, with HB containing DIG-labeled probes (100–500 ng/ml) or radiolabeled probe.
(5 \times 10^6 \text{ cpm/ml}). Following hybridization, the slides were washed twice, for 1 hour and 10 minutes, respectively, in 1x standard saline citrate (SSC; NaCl–sodium citrate) at 48°C, in RNAse solution (5 M NaCl, 1 M Tris-HCl, 0.5 M EDTA, 50 µg/ml RNAseA) for 30 minutes at 37°C, and in consecutive washes at 55°C in 0.5x and 0.1x SSC. The last wash was done at RT. After washing, sections were processed as described by Perez and Caminos (1995). Sections hybridized with radiolabeled probes were coated in Kodak NTB-3 photoemulsion, kept in the dark for 2–6 weeks at 4°C, developed, and fixed. Some slides were counterstained with hematoxylin and eosin. No signal was detected in sections incubated with HB without probe and sections incubated without anti-DIG. Moreover, in positive controls, specific hybridization was observed in hippocampal sections from rat brain, where the expression of neurotrophin receptors has been demonstrated (Merlio et al., 1992; Lindvall et al., 1994).

**RESULTS**

**Distribution of neurotrophin receptors and their ligands in the normal and axotomized tench retinas**

The patterns of immunostaining were specific for each antibody used. We did not observed regional differences in neurotrophin expression between the areas next to the marginal proliferating zone and the more mature part (the central retina); therefore, in the present study, all the data and figures are referred to the central retina. Because the only molecules whose levels changed between intact and lesioned retina were the TrkA receptor and its mRNA, in the description of the results for the rest of the receptors and neurotrophins, we mention only those obtained in normal retina.

**TrkA.** Anti-350 antibody directed against an extracellular domain epitope of TrkA produced moderate immunostaining in the outer nuclear layer (ONL), horizontal and amacrine cells in the INL, and cell bodies in the GCL. In the GCL and inner portion of the INL, a population of one to five cells per section whose size was larger than the rest of the cells showed a stronger immunoreactivity (Fig. 2A). Labeled endfeet and processes of Müller cells and glial cell bodies were also observed spanning the entire thickness of the nerve fiber layer (NFL; Fig. 2C).

Differences in the immunostaining between normal (Fig. 3A) and lesioned (Fig. 3B–D) retinas were observed when using the anti-350 antibody. Seven days after optic nerve crush, the pattern of immunoreactivity obtained was identical to that observed in intact retinas; however, the number of neurons with strongly stained perikarya increased in the GCL (Fig. 3B). Fifteen and 20 days after the lesion, the number of positive ganglion cells increased compared with that seen in retinas analyzed at shorter times after injury. Thirty days after optic nerve crush, the cytoplasm of most ganglion cells was immunopositive, showing very strong labeling for this TrkA antibody (Fig. 3C,F). Ninety days after optic nerve crush, the pattern of immunoreactivity was the same as that in normal retina, and no further changes were observed at any later experimental time examined (up to 210 days after optic nerve crush) (Fig. 3D).

**trkA mRNA.** The trkA mRNA in situ hybridization showed a pattern of distribution in normal tench retina similar to that seen when using immunohistochemistry (Fig. 2A,B). Thus, trkA mRNA was detected mostly in cells localized within the GCL and only occasionally in the INL (Fig. 2B). Between 7 and 30 days after optic nerve crush, an increase in the trkA mRNA expression was also detected (data not shown).

**TrkB.** Labeling in the retina was the same when using antibodies directed to intracellular or extracellular domains of TrkB receptor (Fig. 4). In tench retina, strong immunoreactivity was observed in the photoreceptor
pedicles (Fig. 4A,B), in the INL (at the level of amacrine cells), and in the GCL. Medium and large ganglion cells were immunolabeled in the GCL (Fig. 4A). Vertical processes from Müller cells and ganglion cell fibers were also immunostained in the NFL (Fig. 4A).

**trkB mRNA.** No specific hybridization was observed when radioactive or DIG-labeled mouse or chicken cDNA probes were used.

**TrkC.** When using the anti-Y15E antibody, strong TrkC immunostaining was observed in the ONL and around the cell bodies at all INL levels (Fig. 5A,B). Axon terminals and/or dendrites formed two defined punctated layers within the inner plexiform layer (IPL), and the perikarya of neurons in the GCL and fibers in the NFL were also TrkC immunoreactive.

**trkC mRNA.** An antisense riboprobe corresponding to the intracellular domain of TrkC, which labels cells expressing full-length TrkC, hybridized to a large number of neurons in the ONL, INL, and GCL (Fig. 5C,D). The results were consistent with those obtained by immunohistochemical techniques.

**p75NTR.** Cone photoreceptors were strongly immunolabeled with anti-p75 antibody (Fig. 6A,B) as were most cells in the inner portion of the INL and in the GCL (Fig. 6A). Lightly labeled Müller cell processes were also detected.

**NGF.** Immunostaining for NGF in tench retina was found exclusively in Müller glial processes extending from the INL sclerally to the external limiting membrane (ELM) and vitreally in the IPL and NFL (Fig. 7A,B).

**BDNF.** Strong BDNF immunostaining was observed in Müller cell bodies and in their processes extending from the cell bodies in the INL to the ELM and NFL (Fig. 7C,D). Lightly stained somata were also observed in the INL and GCL.

**NT-3.** All cellular retinal layers were NT-3 immunopositive. The label was detected mainly in the nuclei of the cells (Fig. 7E), and some of these cells also showed stained cytoplasm (Fig. 7F). Glial cells were also NT-3 immunoreactive in the NFL.

**NT-4/5.** A small population of strong NT-4/5-immunostained cells (one to five cells per section) was localized in the inner portion of the INL, IPL, and GCL (Fig. 7G). These cells had immunopositive processes directed toward the IPL. Punctated, horizontal thin processes were also immunostained with anti-NT-4/5 in the NFL as observed running across the optic nerve head to the optic nerve (Fig. 7G). Müller cell processes were also stained in the NFL.

**DISCUSSION**

The present study shows the cellular localization of neurotrophin receptors (TrkA, TrkB, TrkC, and p75) and their ligands (NGF, BDNF, NT-3, and NT-4/5) in the tench retina. The immunodistribution patterns were distinct for...
each neurotrophin receptor and ligand, with some commonly stained elements among them. Moreover, the distribution of most of the neurotrophins and their receptors in tench differed from that found in other species.

Immunohistochemical analysis

Increased immunoreactivity for the TrkA receptor was observed during the regeneration of retinal ganglion cell axons, suggesting that TrkA receptor may be involved in this regeneration process in the tench. Similar results have been found using another antibodies against TrkA (Veciuo et al., 1999). It has been demonstrated that NGF enhances the regeneration of fish retinal ganglion cells (Turner et al., 1981; Yip and Grafstein, 1982); thus, the increase of TrkA in the retinal ganglion cells reported in the present work is consistent with an effect of NGF on the regenerative process. We did not detect an increase in NGF expression in the retina during the regeneration of the retinal ganglion cell axons.

In rat retina, TrkA and NGF were also localized in Müller cell processes (Chakrabarti et al., 1990; Vecino et al., 1998a,b). The role of endogenous NGF has been shown by treatment of retinal ganglion cell cultures from goldfish with anti-NGF, which decreases neurite outgrowth (Turner et al., 1981). Moreover, the effects of exogenous NGF on retinal cells are widely known: it promotes the survival of retinal ganglion cells and optic nerve fibers in rat (Carmignoto et al., 1989), stimulates neurite outgrowth in lesioned optic nerve in goldfish (Turner et al., 1981, 1982; Yip and Grafstein, 1982), and promotes the recovery of retinal ganglion cells from ischemic damage (Siliprandi et al., 1993).

TrkB immunoreactivity was found in amacrine and ganglion cells and was particularly high in photoreceptor pedicles of tench retina. In other fish species, TrkB has been also localized in amacrine and ganglion cells (Cellerino and Kohler, 1997). Although physiological studies have suggested the presence of the TrkB in photoreceptor cells in rat (LaVail et al., 1992; Okazawa, 1994), it has not been detected in these cells in normal rat retina (Jelsma et al., 1993; Zanellato et al., 1993; Rickman and Brecha, 1995). However, a small population of photoreceptor cells in rat retina become immunoreactive to TrkB after ischemic insults (Vecino et al., 1998b). TrkB is a complex receptor, with at least seven distinct trkB transcripts identified in rat brain (Middlemas et al., 1991), six in mouse, and two in zebrafish (Klein et al., 1990; Martin et al., 1995). When using antibodies against both intra- and extracellular domains of the TrkB receptors, we found photoreceptor cells labeled in the tench retina, indicating that at least the full-length form of the TrkB receptor is present in photoreceptors. Moreover, p75 was also localized in photoreceptors. The localization of both Trk and p75 neurotrophin receptors in the same retinal cell type also has been reported in other species when using immunohistochemistry (Carmignoto et al., 1991; Henderson, 1991; Zanellato et al., 1993; Sandel et al., 1994; Rickman and Brecha, 1995). Experimental evidence supports the hypothesis that interactions between high- and low-affinity neurotrophic receptors mediate the internalization and transport of the neurotrophins (von-Bartheld et al., 1996a), and this could also be the case in fish.

Some lines of evidence strongly indicate a protective effect of neurotrophins against degeneration of ganglion cells following optic nerve transection and retinal ischaemia in rat retina (Mey and Thanos, 1993; Mansour-Robaey et al., 1994; Unoki and LaVail, 1994; Weibel et al., 1995; Peñado-Ramón et al., 1996; Sawai et al., 1996; Vecino et al., 1998b). Moreover, ganglion cells survive in culture in response to BDNF (Johnson et al., 1986; Rodriguez-Tébar et al., 1989; Thanos et al., 1989; Castillo et al., 1994), and intravitreal injections of BDNF rescue adult photoreceptor cells from cell death following constant light exposure (LaVail et al., 1992). Recent studies have shown that BDNF regulates early cell death during retinal ganglion cell neurogenesis in chick embryo (Frade et al.,

Fig. 4. A: Photomicrograph of central sections of tench retina labeled with antibodies directed to extracellular epitopes of TrkB. Strong staining was observed at the level of photoreceptor pedicles (arrow), the inner nuclear layer (INL), the ganglion cell layer (GCL), and the nerve fiber layer (NFL). B: High magnification view of positive photoreceptor pedicles. For abbreviations, see list. Scale bars = 50 µm in A, 10 µm in B.
In tench retina but not in rat and chick retina, BDNF is confined mainly to the Müller cells and to cells in the GCL. Considering the effects of BDNF on ganglion cells, the permanent production of BDNF by fish Müller cells, but not by rat Müller cells, may play a role in the capacity of retinal ganglion cells to regenerate their axons in the tench.

We found widespread TrkC distribution in tench retina when using an antibody against the intracellular domain of the chicken TrkC receptor (anti-Y15E antibody). Binding experiments have shown the existence of TrkC in both fish retina (Vecino et al., 1997) and in ferret (Allen-doerfer et al., 1994) retinas. However, in rat retina, TrkC has not been detected (Rickman and Brecha, 1995). TrkC is also a complex receptor with multiple functional isoforms: eight trkC transcripts have been detected in rat and mouse brain (Lamballe et al., 1991; Tsoulfas et al., 1993; Valenzuela et al., 1993; Garner and Large, 1994), and two different TrkC receptors have been detected in zebrafish retina (Martin et al., 1995). The heterogeneity of the TrkC localization across different species may be related to the complexity of this receptor.

NT-3 is widely distributed in most retinal cell types, with a similar pattern in both fish and rat retinas (Vecino et al., 1997).
et al., 1998b). In the present study and in the study by Vecino et al. (1998b), NT-3 immunoreactivity was detected mostly in the nuclei of the stained cells. The nuclear staining was observed with three different anti-NT-3 antibodies (data not show). Nuclear localization has been reported for other neurotrophins and growth factors in some cell types (Yankner and Shooter, 1979; Rakowicz-Szulczynska et al., 1986; Baldin et al., 1990). In tench retina, however, only NT-3 shows nuclear staining. The conserved pattern of expression of NT-3 throughout species could be related to the important functions attributed to this neurotrophin as a factor required during normal development and cell differentiation (De La Rosa et al., 1994; Bovolenta et al., 1996).

The distribution of p75 in both amacrine and ganglion cells in fish contrasts with its distribution in rat, where it is present mostly in Müller cells (Vecino et al., 1998b). The receptor p75 has been suggested to act as a receptor that selectively modulates the biological actions of neurotrophins by interacting with the high-affinity neurotrophic receptors (Ryden et al., 1995; Frade et al., 1997; von Bartheld et al., 1996). Within the tench retina, we found similar cellular localizations for TrkB and p75. We are studying the possible colocalization and functional interactions. The immunodistribution of neurotrophin receptors (Trks and p75NGF) and neurotrophins (NGF, BDNF, NT-3, and NT-4/5) in the tench retina are summarized in Table 1.

**In situ hybridization analysis**

The distribution pattern of specific trk mRNAs in tench retina was comparable with that seen when using immunohistochemistry. This finding supports the specificity of the anti-Trk antibodies employed in the present study. In previous studies, the expression of trkA mRNA was not found in zebrafish retina 6 days after fertilization (Martin et al., 1995), but the possibility of trkA expression at later stages was not excluded. In the present study, we have shown the expression of trkA mRNA in adult fish retina. After optic nerve crush, we observed an increase in the TrkA receptor protein and an increase in the trkA mRNA (data not shown). However, we could not detect trkB mRNA in the adult tench retina with mouse or chick trkB probes. TrkB mRNA has been detected in Xenopus, zebrafish, rat, and avian retinas (Martin et al., 1995; Perez and Caminos, 1995; Hallböök et al., 1996; Cohen-Cory and Fraser, 1994). The sequence of tench trkB may be too divergent from that of the mouse and chiken, thus precluding cross hybridation in the present conditions. The expression pattern of trkC mRNA in the tench retina coincides with the distribution of the TrkC protein.

In conclusion, the neurotrophins and their receptors are differentially distributed throughout the tench retina. NGF and BDNF were detected mainly in Müller glial cells, whereas NT-3 has a broader distribution, and NT-4 is located in a small population of putative ganglion cells. Moreover, the distribution of the receptors is broader than that of the neurotrophins. However, the exact relationship between cells expressing ligands and receptors will require colocalization experiments. Optic nerve lesion induced an increase in TrkA at the protein and mRNA levels during the regeneration period and returned to normal levels when regeneration of the ganglion cell axons was completed. The restricted localization pattern of the neurotrophins and their receptors within the tench retina and

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**Fig. 6.** A: Photomicrograph of the central section of tench retina labeled with antibodies directed to p75NGF. Positive photoreceptor pedicles (arrow) and labeled cells in the inner nuclear (INL) and ganglion cell (GCL) layers are shown. B: High magnification view of positive cone photoreceptors. The photomicrographs were taken from the central retina. For abbreviations, see list. Scale bars = 50 µm in A, 10 µm in B.
Fig. 7. Photomicrographs of neurotrophin immunolocalization in normal tench retina. A: Nerve growth factor (NGF) immunoreactivity. Arrows point to Müller cell processes. B: High magnification view of A showing the Müller cell endfeet in the nerve fiber layer (NFL). C: Brain-derived neurotrophic factor (BDNF) immunoreactivity in the Müller cell bodies (large arrow), with processes from the inner plexiform layer (INL) extending to the external limiting membrane (ELM) and NFL. Weakly positive cells in the INL and ganglion cell layer (GCL; small arrows). D: High magnification view of C showing immunopositive Müller cells. E: Neurotrophin-3 (NT-3) immunoreactivity (NT-3); note that most cell bodies were NT-3 positive. F: Detail of NT-3 immunoreactivity in the INL; note the weakly labeled cytoplasm (small arrowheads) in contrast to the strongly labeled nuclei (large arrowheads). G: NT-4/5 immunoreactivity in a small cell population with large cell bodies (large arrow) and in fibers (small arrows) in the GCL and NFL running over the optic nerve head (onh). For abbreviations, see list. Scale bars = 50 µm in A, C, E, G; 10 µm in B, D, F.
the changes observed in TrkA receptor during the regeneration of the ganglion cell axons may reflect the importance of these molecules in retinal function in fish retina.

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LITERATURE CITED


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