Distribution of Transcript and Protein Isoforms of the Synaptic Glycoprotein Neuroplastin in Rat Retina

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PURPOSE. To examine the expression and localization of the neuroplastins (np), two synapse-enriched members of the immunoglobulin (Ig) superfamily of cell-adhesion molecules, in the developing and adult retina and optic nerve.

METHODS. Expressions of the two isoforms np55 and np65 and carboxy-terminal splice variants were investigated by immunocytochemistry, Western blot analysis, RT-PCR, and in situ hybridization.

RESULTS. Immunoreactivity for both neuroplastins was confined to the two synaptic layers of the retina: the inner (IPL) and outer plexiform layer (OPL). Significant overlap was found in staining at synaptic structures with synaptophysin. A large proportion of immunoreactivity for both isoforms, however, was of perisynaptic origin. In situ hybridization studies were suggestive of a pre- and postsynaptic localization of np65 in the OPL. Transcripts for np55 were already present at birth in the inner retina, but the hybridization signals increased during postnatal development. Np65 transcripts and immunosignals appeared at later developmental ages, concomitant with synapse formation in the OPL. Several C-terminal neuroplastin cDNA clones harbor an insert of 12 bp, coding for four amino acids (DDEP) in the intracellular domain of neuroplastins. Splice isoforms containing the insert exhibited a developmental expression pattern similar to that of np55; however, both neuroplastins could harbor the C-terminal insert. Neuroplastins were also detected in optic nerve homogenates. RT-PCR and blockade of axonal transport by nerve crush confirmed transcript and protein expression in optic nerve tissue.

CONCLUSIONS. The findings suggest a role for neuroplastins in cell adhesion in the plexiform layers during histogenesis, as well as in maintenance of connections between specific cellular structures. (Invest Ophthalmol Vis Sci. 2001;42:1907–1914)

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Cell adhesion is a fundamental prerequisite for the genesis, maintenance, and plasticity of synaptic contacts throughout the nervous system. These processes are mediated by a variety of neural cell adhesion molecules (CAMs), which are expressed in a highly segregated spatial and temporal manner. Np65 and np55 are isoforms of neuroplastin, a glycoprotein of the synaptic membrane protein fraction of rat brain with apparent molecular masses of 65 and 55 kDa, respectively.¹,² Deglycosylation studies have revealed that the protein cores of np55 and np65 have molecular masses of 28 and 40 kDa, respectively.¹ They were originally identified as gp65 and gp55 and biochemically characterized using the monoclonal antibody (mAb) Smgp65.¹,³ Both molecules belong to the immunoglobulin (Ig) superfamily of CAMs and behave as integral membrane proteins. Np65 contains three Ig domains and np55 two.² They are arise by alternative splicing from the same primary transcript and differ by one N-terminal Ig domain, which contains no N-linked glycosylation site.² Fractionation of brain protein homogenates has shown that np65 is enriched in the postsynaptic density fraction of synaptic junctional proteins, whereas np55 is virtually absent from this fraction.¹,³ Although not exclusively localized at the synapse, it is likely that np65 is involved in adhesion of synaptic membranes.

Whereas np65 is primarily expressed in the brain, np55 is widely distributed in many tissues and occurs in multiple glycoforms.² The transcript and protein isoforms of the neuroplastins are largely unknown. Members of several CAM families have been localized to synaptic junctions and are thought to mediate synapse formation and stabilization during development and to be required for activity-dependent long-term restructuring of synapses.³ Our recent evidence suggests that np65 plays a role in cell adhesion mechanisms involved in long-term, activity-dependent synaptic plasticity.⁴ A detailed analysis of the cellular and subcellular localization of neuroplastins in the central nervous system, however, is still needed.

The retina is particularly well suited for this type of study, because its cell types and synaptic connections are organized into different layers and its cytoarchitecture is well understood.⁵ Moreover, in the rat retina nerve cell maturation and interneuronal connectivity occur postnatally, which makes this model particularly useful for developmental studies.⁶,⁷ Therefore, we analyzed the developmental and adult localization of np65 and np55 transcripts and protein in rat retina. In addition we investigated the expression pattern of a processing variant of neuroplastins that was detected originally in np55 transcripts and codes for a four-amino-acid (DDEP) insertion into the C-terminal cytoplasmic domain of the protein.² We investigated whether only np55 or both neuroplastins contain this insertion and the pattern of expression of these splice variants in brain and retina.
Localizaton of neuroplastin probes and antibodies

METHODS

Animals and Surgery

Male Sprague-Dawley rats from the local breeding facilities of the Leibniz Institute were used for all experiments. Animals were kept on a 12-hour dark-light cycle with food and water available ad libitum. All rats were killed 4 hours after onset of the light period, and retinas of at least three animals for each developmental time point were dissected and processed for in situ hybridization, Western blot analysis, and immunohistochemistry. Optic nerve crush was performed as described previously.\(^6\) Animal experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the regulations for animal experiments of the federal government of Germany.

In Situ Hybridization

In situ hybridization of rat retinal and brain cryostat sections was performed as described previously.\(^10,11\) The position of the oligonucleotide probes and their complementary location on the neuroplastin cDNA is shown in Figure 1. Various rat brain cDNA libraries were screened for overlapping neuroplastin cDNA clones.\(^2\) Sequence analysis of these clones revealed that numerous clones contained an insertion of 12 bp encoding the amino acids DDEP in the cytoplasmic tail of neuroplastin.\(^2\) Specific oligonucleotides for isoforms either harboring or without the C-terminal insertion were synthesized, and in situ hybridization studies of rat brain and retina cryostat sections were performed. The nucleotide sequences of the probes were as follows: np65: 5’-gtgctgtgaagactctggcggctgtctgtgacac-3’; np65/55: 5’-ctctgctctgctgtgctgactgctgtgactgctgtgactgctgtgactgctg-3’; np55: 5’-gtgctgtgaagactctggcggctgtctgtgacac-3’; DDEP+: 5’-cattggcccggactttgctgtgctgtgactgctgtgactgctgtgactgctg-3’; and DDEP−: 5’-gttctttcactgactgactgactgactgactgactgactgactgactgactg-3’.

Specificity of labeling was checked by a variety of control procedures including pretreatment with RNase A (Promega, Mannheim, Germany) and use of a sense probe. Some alternate sections were stained with 0.125% cresyl violet to reveal the cellular pattern and layering of the retina.

Isoform-Specific Neuroplastin Antibodies and Western Blot Analysis of Brain, Retinal, and Optic Nerve Protein Homogenates

Production and characterization of rabbit polyclonal antisera AS Ig1 and AS Ig2-3 directed against the np65-specific and the two common Ig domains, respectively, have been described previously.\(^6\) The mouse mAb SMgp65 was generated against a ConA-binding glycoprotein fraction prepared from rat brain synaptic membranes and recognizes both neuroplastin isoforms.\(^5\) Retinal homogenization and Western blot analysis were performed essentially as described previously.\(^5,10\) Homogenates from retinal sublayers were prepared from cryostat sections of retinal flatmounts.\(^12\) The first two sections (40 μm each) were found to contain mainly the optic nerve fiber layer, retinal ganglion cell layer (GCL), and the inner plexiform layer (IPL). The next two sections contained mainly the inner nuclear layer (INL) and the outer plexiform layer (OPL), whereas the last three sections predominantly contained cells from the outer nuclear layer (ONL).

Immunostaining of Rat Retinal Paraffin and Cryostat Sections

Staining of retinal paraffin sections was performed as described previously.\(^13\) The slides were incubated for 1 hour with 5% normal goat serum (Sigma, Deisenhofen, Germany) in 10 mM PBS (pH 7.4), followed by an overnight incubation with mAb SMgp65 (dilution 1:800). The slides were washed three times for 5 minutes in 10 mM PBS and incubated with the secondary antibody by the ABC method (Vectastain; Camon, Wiesbaden, Germany), according to the manufacturer’s protocol.

Immunostaining of cryostat sections was performed as described previously.\(^14\) For immunofluorescence experiments, vertical retinal

Figure 1. Schematic representation of the Ig and cDNA structures of np65 and np55. Positions of PCR primers and oligonucleotides used for in situ hybridization are indicated. In addition, the epitopes for the monospecific (ASlg1), the monoclonal (SMgp65), and rabbit polyclonal (ASlg2-3) antisera are depicted.

Nomenclature

- **ASlg1**: Antibody specific for np65
- **ASlg2-3**: Antibody recognizing both np65 and np55
- **mAb SMgp65**: Mouse antibody recognizing both np65 and np55
sections (10 μm) were incubated for 1 hour in blocking solution, containing 10% normal goat serum (NGS) and 1% bovine serum albumin (BSA) in 0.01 M PBS (pH 7.4), followed by an overnight incubation with the primary antibody, either AS Ig1 (dilution 1:500) or AS Ig2-3 (dilution 1:1000), diluted in incubation solution containing 3% NGS and 1% BSA in PBS. After several washes in PBS, the sections were incubated for 2 hours with secondary antibody (goat anti-mouse IgG and goat anti-rabbit IgG, either conjugated to CY2 or to CY5 (carboxymethylindoxylicyanine; Dianova, Hamburg, Germany) diluted in incubation solution. Mouse monoclonal anti-synaptophysin (Sigma) was diluted 1:2000. Confocal laser scanning microscopy was performed as described previously. Control experiments were performed by omission of the primary antibody and incubation with the rabbit preimmune serum.

RNA Extraction and RT-PCR

Total RNA was extracted from adult rat retina and optic nerve using a commercially available kit (RNeasy; Qiagen, Hilden, Germany). The following oligonucleotides were used as PCR primers (see also Fig. 1): np65 forward (np65F), 5′-gaagcgccgtgacctgaaac-3′; np55 forward (np55F), 5′-cgcgtcagaacagcggaaaga-3′; np65 and np55 common reverse (npR), 5′-catggccggcagcaggaacaaag-3′; np65/55DDEP− reverse (npD−R), 5′-cttgcccggtctctcatttgaagagccgcggtgtctacac-3′. PCR was initiated by adding 30 picomoles of each forward primer and 60 picomoles of each reverse primer to 2.5 μl 10× PCR buffer (Clontech, Heidelberg, Germany), 200 μM dNTPs, and 2.5 units Taq polymerase (Advantage; Clontech) to 1, 10, or 100 ng cDNA. The reaction mixture was brought to 25 μl with H2O before the cDNA was denatured at 95°C for 1 minute. Annealing and extension temperatures were 55°C and 72°C, respectively, for 1 minute each. Denaturing temperature was set at 95°C for 1 minute. After 38 cycles, 8 μl of the sample was separated on a 1.5% agarose gel including ethidium bromide to visualize the bands.

RESULTS

Expression of Transcripts of Neuroplastin Isoforms with or without the DDEP Insertion

Transcripts encoding the DDEP insertion in the cytoplasmic tail of neuroplastin (Fig. 1) have been detected in np55 cDNA clones. A PCR strategy was designed to detect whether this insertion is present also in the 65-kDa isoform of neuroplastin. To this end PCR primers were designed that specifically recognized DDEP+ or DDEP− isoforms at the 3′ end and np55 or np65 at the 5′ end. The localization of primer oligonucleotides is indicated in Figure 1. As shown in Figure 2, transcripts for all four isoforms—np55 DDEP−, np55 DDEP+, np65 DDEP−, and np65 DDEP+—were expressed in both brain and retina. It should be noted that the strategy was not designed to quantify the transcript levels of the individual isoforms.

In situ hybridization studies were performed to assess the distribution of the different isoforms in the brain. As reported previously, 6 np65 is more abundant in forebrain regions and less abundant in the cerebellum than np55 (Fig. 2). These different transcript levels are also reflected by different protein levels of both isoforms as evidenced by Western blot analysis of cerebellum and basal ganglia total protein homogenates. 1,3 The expression patterns of DDEP+ and DDEP− isoforms also showed some variation (Fig. 3). DDEP+ splice variants were more prominently found in the cerebellum. The high level of np65 relative to np65 in the cerebellum suggests expression is primarily np55SDDEP+ in this region. DDEP− isoforms were found throughout the brain including hippocampus and forebrain regions (Fig. 3). Both np65 and np55 transcripts are thought to contribute to labeling obtained with these oligonucleotides.

Using the common probe that detects all splice variants of neuroplastin, transcripts were detected in all three cell layers of the adult retina (Fig. 4). Hybridization signals were homogeneously distributed, indicating expression of np65 and np55 in the majority of retinal cell types. In situ hybridization with the np55-specific probe produced hybridization signals only in the INL and the GCL. Silver grains covered all sublaminae of the INL, suggesting that amacrine, bipolar, and horizontal cells express np55 (Fig. 4). Experiments using the np65-specific probe showed that cells in all three cell layers were labeled.
(Fig. 4). The localization of transcripts with probes for the two N-terminal splice variants was in agreement with the signal found with the common probe. Transcripts for the C-terminal processing variants were localized to all retinal nuclear layers with slightly stronger hybridization signals for DDE-- variants (Fig. 4).

**Developmental Regulation of Neuroplastin Transcripts in the Retina**

To elucidate whether the expression of neuroplastin isoforms correlates with specific stages of retinal development, hybridization experiments were performed with tissue obtained at postnatal day (P)1 to P14 (Fig. 5). Np55 was found to be expressed already at P1 in the cytoblast layer (CBL; also referred to as ventricular zone) and primordial GCL (Fig. 5). At this stage, no np65 hybridization signals above background were observed. In the INL and ONL a faint label with the np65 specific probe first appeared at P4 (Fig. 5). At P6 this label was much stronger, covering mainly the ONL and the outer half of the INL (Fig. 5). At this time point, the synaptic connections in the OPL have formed, and synaptogenesis of ribbon synapses between photoreceptors and bipolar cells is occurring in albino rats. Ribbon synapses in the IPL are formed at P11 to P13. Faint in situ label for np65 was present in the GCL at this stage of retinal development, coinciding with maturation of synapses in the INL and eye opening (Fig. 5). Expression of DDE+ and DDE-- splice variants was essentially similar to those of np65 and np55 at all stages of retinal development (data not shown).

**Neuroplastin Protein Expression in the Adult and Early Postnatal Retina**

Western blot analysis using mAb SMgp65 and a polyclonal antiserum AS Ig2-3 directed against the common region of np65 and np55 confirmed that both glycoproteins were expressed in the adult rat retina (Fig. 6). Moreover, the np65-

**Figure 3.** In situ hybridization of neuroplastin isoforms in the adult brain. Horizontal cryostat sections were hybridized with np65-, np55-, DDE--, and DDE+--specific probes (see Fig. 1 for the position of the probes).

**Figure 4.** In situ hybridization of neuroplastin isoforms in the adult rat retina. Retinal cryostat sections were hybridized with np65-, np55-, DDE--, and DDE+--specific probes (see Fig. 1 for the position of the probes) and the np65 and np55 core probe, which detects all splice isoforms. Scale bar, 50 μm.

**Figure 5.** In situ hybridization of neuroplastin isoforms during retinal development are depicted in the left panels. Np55 hybridization signals were found to be diffusely distributed early in development but became restricted to the INL and GCL in the second postnatal week. Hybridization signals similar to those of the np65 and np55 core probe were seen with DDE-- and DDE+--specific probes (not shown). In the right panels, immunostaining of retinal cryostat sections with ASlg1 (np65) and ASlg2-3 (np65/55) is depicted at different stages of postnatal development. In contrast to the diffuse distribution of immunosignals with ASlg-2-3 the appearance of np65 immunoreactivity largely correlated with the histogenesis and synapse formation in OPL and IPL. In the first and fourth rows, sections counterstained with cresyl violet were inserted to facilitate morphologic orientation at each time point during retinal development. Scale bar, 50 μm.

specific antiserum AS Ig1 detected only one protein band migrating at the molecular weight of np65 (Fig. 6). Thus, all antibodies used in this study were specific for either one or both neuroplastin isoforms.
With monoclonal and polyclonal antibodies recognizing both neuroplastin isoforms, immunoreactivity was found in both synaptic layers, IPL and OPL, in rat retinal paraffin sections. A similar staining pattern was found with the monospecific np65 polyclonal antiserum (Fig. 7). Immunofluorescence studies with AS lg2-3 and AS lg1 confirmed the presence of intensely labeled structures in the OPL and IPL (Fig. 7). In the IPL, immunoreactivity was concentrated in one thin layer (Fig. 7), surrounded by more diffuse label. Double-immunofluorescence confocal laser scanning microscopy with an antibody against the presynaptic marker protein synaptophysin and AS lg1 showed that immunofluorescence for np65 was colocalized to a large degree with immunoreactivity of this synaptic marker in the OPL and INL (Fig. 7). It should be noted, however, that np65 and np65/np55 immunoreactivities were broader and more diffusely distributed in the OPL. This probably indicates an additional perisynaptic localization. Unfortunately, although we tried several different protocols, all neuroplastin antibodies used in this study were found to be unsuitable for ultrastructural studies using electron microscopy.

To further substantiate the immunostaining data, retinal flatmounts were cut on a cryostat, and the retina was divided into three parts: sections containing mainly GCL and IPL, or INL and OPL, or ONL. Western blot analysis of these preparations showed abundant expression of both isoforms in the inner retina and the OPL with very low levels in the ONL (Fig. 8). Most interesting, np65 was found to be much more abundant in the preparation containing the OPL photoreceptor synapse fraction than np55 (Fig. 8). Synaptophysin was used as a synaptic marker protein to compare the content of synaptic structures with that of neuroplastins in all three preparations (Fig. 8).

In agreement with the in situ hybridization experiments we found np65 and np55 immunoreactivity, as detected with AS lg2-3, to be diffusely distributed in the CBL and GCL (Fig. 5). This distribution was altered in the second postnatal week with prominent label in both synaptic layers (Fig. 5), although cellular staining was still present at this stage and in mature retinas (Figs. 5, 7). Stainings with the monospecific AS lg1 antiserum confirmed the presence of np65 immunoreactivity in the OPL and IPL during retinal development and demonstrated a correlation of histogenesis of the OPL at P6 and synapse formation in the IPL in the second postnatal week with the first appearance of np65 immunosignals restricted to these layers (Fig. 5).

**Neuroplastin Expression in the Optic Nerve**

Immunoblot analysis revealed neuroplastin-immunoreactive bands in optic nerve protein preparations, indicating that both isoforms were present in the optic nerve. To identify the origin and potential regulation of neuroplastin expression, a crush of the optic nerve was performed to interrupt axonal transport. After nerve crush, immunoreactivity for both neuroplastins did not accumulate in the proximal part of the optic nerve, which was still in continuity with retinal ganglion cells. The immunoreactivity in the distal part of the nerve was also not affected (Fig. 9). RT-PCR experiments showed that transcripts for both neuroplastins were present in the optic nerve (Fig. 9).

**Discussion**

In this study we localized immunoreactivity for the 65- and 55-kDa isoforms of neuroplastin to the synaptic layers of the retina. Corresponding transcripts were found to be differentially distributed with predominant expression of np65 transcripts in the outer retina, whereas both np55 and np65 transcripts were present in the inner retina. In particular, np65 appeared to be associated with synaptic structures in the OPL—that is, it colocalized with the presynaptic marker protein synaptophysin. Immunoreactivity of the neuroplastins in the INL was more diffusely distributed. Thus, although not restricted to synapses, neuroplastins are most probably found at both conventional and ribbon synapses of the retina. Moreover, we provide evidence for the first time for the expression of neuroplastins in central nervous system glia. Previous studies have shown no evidence for glial localization of the neuroplastins in rat brain, and neither np55 nor np65 is expressed by cultured astrocytes.5,6

The immunoreactivity for both neuroplastins was confined to the two synaptic layers of the retina. Although, due to methodologic limitations, a direct localization at the synapse could not be shown with immunoelectron microscopy, several lines of evidence suggested that at least np65 is localized at ribbon and at conventional synapses. First, we found a clear colocalization of neuroplastins with a synaptic marker protein in both synaptic layers. Second, the appearance of np65 transcripts and immunosignals during retinal development coincided with the period of formation of ribbon synapses in the OPL. In addition, the overlap of synaptophysin with np65 immunoreactivity in the OPL strongly suggests that neuroplastins are CAMs situated in the synaptic cleft of ribbon synapses. Neuroplastin immunoreactivity was broadly distributed, which probably indicates that either np55 or both neuroplastins are also found in horizontal cell synapses and perisynaptic regions. In the IPL, neuroplastin immunoreactivity was more diffuse. Thus, it seems that np65 is a component enriched in synaptic structures of the retina, which is in accordance with the previous finding that the 65-kDa, but not the 55-kDa isoform is enriched in rat forebrain postsynaptic density preparations.5

The localization of np65 transcripts in the ONL and INL suggests both a pre- and postsynaptic localization of the protein in ribbon synapses of the OPL. Because most of the np65
immunoreactivity was found in the OPL it has to be assumed that the translated transcript in photoreceptor cells is localized at presynaptic structures. Of interest in this respect are recent binding experiments in which neuroplastin-Fc chimeric proteins were used to show that constructs containing the Ig domains 1 to 3 or only the np65-specific Ig domain 1, but not constructs containing the two common Ig domains, mediate homophilic adhesion. It is therefore conceivable that np65 at ribbon synapses binds to its postsynaptic counterpart expressed by bipolar cells. Taken together, the available evidence is therefore suggestive of a role of np65 in adhesion between the pre- and postsynaptic nerve endings in the OPL, possibly by a homophilic mechanism.

Although cell differentiation and layer formation of the retina appear to advance in an inside-to-outside sequence with cell birth of ganglion cells first and appearance of photoreceptor cells last, the maturation of synaptic structures proceeds from the outer to the inner layers of the retina. Synaptogenesis of conventional amacrine synapses is first apparent at the end of the first postnatal week and ribbon synapses formed between bipolar and ganglion cells in the IPL are found at P5 with increasing numbers at P12 or P13.19 Ribbon synapses in the OPL, however, are already present at P5 in pigmented rats and at P6 in albino rats, which coincides with the formation of this synaptic layer. In contrast to np55, the transcript levels of np65 are regulated during retinal development in a cell-specific manner.

**Figure 7.** Immunostaining of adult retina with different neuroplastin antibodies. (A, B) Immunostaining with the mAb Snnp65 (A) and rabbit polyclonal ASIg2-3 (B) of rat retinal paraffin-embedded sections. Strong labeling was found in the OPL and IPL. Two prominent immunoreactive bands were detected in the IPL. (C) At higher magnification the labeling was found to be concentrated at one membranous substructure in the OPL (arrows). (D–I) Double immunofluorescence labeling in the IPL and OPL with a synaptophysin (D, G) and the rabbit monospecific ASIg1 (E, H) antibody. Labeling with the monospecific np65 antibody indicated localization of this splice isoform in both synaptic layers (E, F). Double-immunofluorescence confocal laser scanning microscopy show a large overlap of synaptophysin and np65 fluorescence signals in both synaptic layers (F). At higher magnification a perisynaptic localization of np65 immunoreactivity also became evident (I, arrows). Scale bar, (A, B, D–F) 50 μm; (C) 10 μm; (G–I) 5 μm.

**Figure 8.** Western blot analysis of protein preparations from the inner and outer retina with a polyclonal neuroplastin antibody. (A) The relative intensity of immunoreactive bands for np65 compared with np55 were found to be stronger in mainly protein preparations containing the INL+OPL part of the retina than in those containing mainly GCL+IPL. (B) The enrichment of synaptic structures was demonstrated by the presence of synaptophysin immunoreactivity in GCL+IPL and INL+OPL and only negligible amounts in the part containing mainly the ONL. Equal amounts of protein (50 μg) were loaded in each lane.
It is striking that the transcript expression of np65 in the ONL and INL correlated with the formation of ribbon synapses in the OPL, whereas no correlation with retinal synaptogenesis was found for the expression of np55. Moreover, np65 immunoreactivity was also prominent in both synaptic layers during development and, most interesting, immunosignals were first detected at developmental stages correlating with synaptogenesis in the OPL and IPL. Thus, it seems that specifically np65 could be an early cell adhesion component during synapse formation. Moreover, it is tempting to speculate that a homophilic interaction of pre- and postsynaptically situated np65 is involved in the formation of these synapses.

At present few of the molecules involved in adhesion events at conventional and ribbon synapses in the retina have been identified. Of the CAMs localized to synaptic junctions to date, only NCAM-180 and cadherins have been immunolocalized in the retina. The distribution of NCAM-180 was found to be largely similar to those of np65 and np55, whereas cadherin expression was found to be more confined to certain retinal cell populations. In addition, the glycoprotein β-dystroglycan, which is suggested to play a role in cell–cell contacts, has been identified in the OPL and nerve fiber layer of the retina. Similarly, β-dystroglycan seems to have a much more restricted expression pattern compared with the neuroplasins. It is reported to be associated with the more proximal lateral wall of the photoreceptor synaptic cavity that projects into the postsynaptic dendritic complex. The more widespread and persynaptic localization of a significant fraction of np65 and np55 in the OPL suggests that its function is probably less restricted to specific aspects of stabilization of synaptic morphology or clustering of proteins to microdomains, which have been proposed for β-dystroglycan. Thus, a broader function in histogenesis and maintenance of cellular contact in synaptic regions, possibly comparable to those of NCAM-180, has to be assumed for neuroplasins.

A C-terminal splice variant of neuroplasins was identified in retina and brain. The broad expression pattern of both isoforms and the results of the RT-PCR experiments indicated the presence of the DDE insertion in both np65 and np55. It is therefore unlikely that this insertion is specific for neuroplasins that are localized to synapses. The function of the four-amino-acid insertion remains elusive, but because they are situated in the intracellular domain of neuroplasins, it is conceivable that they are involved in mediating a protein–protein interaction that may be important in intracellular signal transduction or protein localization. Further studies are warranted to identify such protein interactions and to determine whether the C-terminal insertion renders this interaction specific for one splice isofrom.

With RT-PCR and Western blot analysis, we confirmed the expression of both neuroplasins in rat optic nerve. The expression was found to be unaltered after nerve crush, indicating that the protein is not transported in the optic nerve but is synthesized by optic nerve glia. The levels of protein were substantially lower in retina and brain. Because the antibodies used in this study were found to be unsuitable for electron microscopy, the functional role of these molecules could not be addressed. However, it would be of interest to elucidate whether a cell adhesion contact between glia cells and optic nerve axons is mediated in part by neuroplasins.

In summary, we have provided evidence for a synaptic localization of neuroplasins in rat retina. In particular, np65 could play a role in cell adhesion between photoreceptors and the postsynaptic complex formed by horizontal and bipolar cells. Thus, it seems likely that neuroplasins belong to the group of molecules that have been implicated in cell adhesion in the plexiform layers during histogenesis as well as in maintenance of connections between specific cellular structures.

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