Identification of a Nervous Tissue–Specific Chondroitin Sulfate Proteoglycan, Neurocan, in Developing Rat Retina

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PURPOSE. To identify the expression of neurocan, a nervous tissue–specific chondroitin sulfate proteoglycan, in retina and to elucidate its changes during development.

METHODS. Expressional changes of neurocan mRNAs in developing rat retinas were investigated by a semiquantitative reverse transcription–polymerase chain reaction (RT–PCR). The localization and characterization of neurocan core proteins were also investigated with the use of Western blot analysis and immunohistochemistry.

RESULTS. Gene expression of neurocan was identified in retinas by RT–PCR. Semiquantitative analysis using Southern blot analysis revealed that mRNA expression for neurocan increased at increasing postnatal stages and that it reached its peak around postnatal day 7 (P7). Immunohistochemical studies demonstrated that in differentiating rat retinal (neuroblast) cells weak neurocan immunoreactivities were observed throughout the retina on embryonal days 14 (E14) and E16. During the early postnatal period, the immunoreactivities became most conspicuous in the inner and outer plexiform layers on P7 through P14. In adult retinas, only faint immunostaining was detected. Immunoblot analysis showed two positive bands of 220- and 150-kDa core glycoproteins after treatment with chondroitinase ABC. Further immunoblot analysis revealed that the expression of these two immunolabeled variants was regulated differently during retinal development.

CONCLUSIONS. The temporal and spatial regulation of expression of neurocan and its proteolytic variant during retinal development suggest that it may play a role in differentiation and neural network formation. (Invest Ophthalmol Vis Sci. 1999;40:2350–2359)

Proteoglycans are some of the major constituents of the extracellular matrix and are composed of a core protein molecule to which glycosaminoglycans (GAGs) are covalently linked as side chains.1,2 They are classified into several GAG side chains. Varner and associates3,4 have clearly demonstrated, by histochemical and biochemical studies, the presence of chondroitin sulfate and heparan sulfate GAGs on the basis of their core side chains. Since then, biological activities of proteoglycans have been shown to elicit an alteration in neurite outgrowth, neural cell adhesion, and differentiation.5,6 Accordingly, characterization of retinal proteoglycans is necessary to better understand both developmental and pathologic processes. Knowledge about proteoglycan core proteins in the retina is limited.7,8 However, it is known that a nervous tissue–specific proteoglycan, neurocan, is the major proteoglycan in the brain9 and that it regulates neurite outgrowth and cell adhesion in neural tissues.10,11 It is also possible that neurocan plays a pivotal role in formation of the neural network. However, to date, there are no conclusive data regarding the expression of neurocan in the retina. It has been reported that a neurocan-derived C-terminal product (also called chondroitin sulfate proteoglycan [CSPG]-150 or neurocan-C) is created in rat brain by proteolytic processes and that it ultimately becomes the major form and has biological activities similar to full-length neurocan.12 In a previous study, a monoclonal antibody (MAb 1G2) recognizing both a 220-kDa glycoprotein (full-length neurocan) and a 150-kDa glycoprotein (C-terminal half proteolytic product of neurocan) was raised.13 Herein, we report on the gene expression and localization of neurocan core protein in the retina and show alterations in the distribution and amount in the developing retina.
METHODS

Retinal RNA Isolation and cDNA Synthesis
Wistar rats at various stages of development were killed by intraperitoneal overdose injection of pentobarbital. After enucleation of the eyes, neural retinas were removed by scissors and forceps under an operating microscope. Total RNA was isolated from the retinas by the acid guanidium thiocyanate-phenol–chloroform extraction method. The extracted RNA was used to synthesize template cDNAs for subsequent reverse transcription-initiated–polymerase chain reaction (RT–PCR) experiments with the use of reverse transcriptase (Super Script RNase H-Reverse Transcriptase; GIBCO–BRL, Life Technologies, Gaithersburg, MD). All animals were given water and food ad libitum. All studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

PCR and Subsequent Southern Blot Analysis
PCR was carried out by the method of Saiki et al., slightly modified as described previously. In PCR experiments for the neurocan gene, the following conditions were used: denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds, and polymerization at 72°C for 1 minute for 40 cycles. Primers used in this study were designed from N terminus of the reported sequences of rat neurocan gene, sequences of the sense and the antisense primers were ACCTGCTGTCGTCTCAGGATG and TTGGCCTGTGCCGGGGATA, respectively. Possible contamination of the genomic sequence was determined by running parallel control experiments without the use of reverse transcriptase. Rat brain RNAs were used as positive controls. After the PCR, the products were separated by 2% agarose gel electrophoresis and then subjected to Southern blot analysis. In Southern blot analysis, the amplified products were separated by 2% agarose gel electrophoresis and transferred to a membrane, Hybond-N+ (Amersham, Buckinghamshire, England) by the capillary transfer method with 20× SSC. Synthesized internal oligonucleotide probe (the sequence, TCGTTGGAAGGAAATCGTGC sense and ACCAGACAGCAGTGGTGTGTTG antisense) was labeled by ECL 3'-oligolabeling and detection systems (Amersham) to confirm the identity of the PCR product and to exclude the nonspecific bands.

Semiquantitative RT–PCR
Extraction of total RNA from rat retinas and creation of first-strand cDNA were performed as described above. cDNA concentration was normalized to β-actin gene expression in a manner similar to that described previously, using specific primers to β-actin: AGCTGAGGAGGAAATCGTGC (sense) and ACCAGACAGCAGTGGTGTGTT (antisense). For PCR experiments, the following conditions were used: denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds, and polymerization at 72°C for 1 minute for 19 cycles (β-actin primers) or 29 cycles (neurocan primers). After the reaction, the products were separated by 2% agarose gel electrophoreses as described above. To investigate relative levels of neurocan gene expression, a semiquantitative analysis was carried out using Southern blot analysis after RT–PCR. Optical densities of hybridizing bands were measured by a Power Macintosh G3 computer (Apple, Cupertino, CA) and NIH Image 1.59 software. A standard curve was generated from the optical densities of hybridizing bands from serial dilutions of template cDNAs, and the linearity of the created standard curve among the selected concentrations was confirmed. The relative levels of mRNA expression were calculated as a ratio to the control (adult rat retina on postnatal day 42 [P42]).

Preparation of Sections for Immunohistochemistry
Embryonal and postnatal Wistar rats were killed by intraperitoneal overdose injection of pentobarbital, and the eyes were enucleated. The enucleated eyes were fixed for 2 hours at 4°C with 4% paraformaldehyde in phosphate-buffered saline (PBS) with gentle shaking, washed for 5 minutes in PBS, then gently shaken overnight at 4°C in 30% sucrose/PBS before freezing on powdered dry ice. Sections (16 μm) were cut using a cryostat and collected onto silanized slides (DAKO JAPAN, Kyoto, Japan).

Immunohistochemistry
Retinal sections were circled with a PAP pen (DAKO Pen; DAKO JAPAN) to form a hydrophobic barrier to solutions, fixed for 30 minutes in 3% paraformaldehyde/1% sucrose/1 mM magnesium chloride hexahydrate/0.1 mM calcium chloride dihydrate/PBS, then rinsed twice for 3 minutes each in PBS. Slides were incubated in cold methanol (−20°C) for 15 minutes and then washed three times for 3 minutes each in PBS. Sections were covered for 15 minutes with 50 mM glycine in PBS, then rinsed for 3 minutes in PBS before covering each slide for 1 hour with blocking solution (2% bovine serum albumin [BSA]/2% horse normal serum/2% goat normal serum in PBS). After slides were washed for 3 minutes in PBS, sections were incubated overnight at 4°C in a humidified chamber with primary antibody. The specificity of the anti-neurocan monoclonal antibody (MAb 1G2) has been described previously. Sections were rinsed three times for 3 minutes each in PBS to remove the primary antibody. Sections were then incubated for 1 hour at room temperature with the secondary antibody, fluorescein-conjugated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA), and washed six times for 3 minutes each in PBS. Sections were mounted in Vectashield (Vector Laboratories), and the slides were examined under a confocal microscope LSM410 (Carl Zeiss, Oberkochen, Germany). Also, in an effort to identify the cell origin of the neurocan-expressing cells in the retina, in addition to MAb 1G2, anti-vimentin IgM and anti-S-100β polyclonal antibodies were used for double-staining technique.

Sample Preparation for Western Blot Analysis
Retinal tissue (30 mg wet weight) at embryonal and postnatal stages was homogenized in 50 μl ice-cold PBS containing 10 mM N-ethylmaleimide (NEM), 20 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride (PMSF). The homogenate was mixed with 200 μl of 20 mM Tris–HCl buffer (pH 7.5) containing 2% sodium dodecyl sulfate (SDS), 10 mM NEM, 20 mM EDTA, and 2 mM PMSF and boiled for 5 minutes. The concentration of an aliquot (10 μl) of the boiled solution was measured by Bio-Rad DC protein assay (Bio-Rad Laboratories, Tokyo, Japan). A 100 μl aliquot (200 μg) of the solution was mixed with 300 μl of 95% ethanol containing 1.3% potassium acetate and incubated for 1 hour on ice followed by centrifugation at 14,000 rpm for 15 minutes. The pellet was washed with 400 μl of 70% ethanol containing 1.0% potassium acetate...
and recentrifuged. Finally, the pellet was dried using the vacuum pump.

**Chondroitinase Treatment**

Protease-free chondroitinase ABC (Seikagaku, Tokyo, Japan) was used to remove the chondroitin sulfate chains from the core protein. To the pellet was added 119 μl of distilled water, followed by the addition of 15 μl of Tris-HCl buffer (1 M, pH 7.5) including 0.3 M sodium acetate, 4 μl of inhibitor mixture-I (20 mM PMSF/7.2 mM pepstatin), 8 μl of inhibitor mixture-II (0.1 M EDTA/0.1 M NEM), and 4 μl of the enzyme solution (0.01 U/μl). The mixture (total 150 μl) was incubated at 37°C for 2 hours. The enzyme reaction was stopped by adding 450 μl of 95% ethanol containing 1.3% potassium acetate. The mixture was then centrifuged at 14,000 rpm for 15 minutes, and the precipitated material was used as the sample for immunoblot analysis.

**Immunoblot Analysis**

To each precipitated material was added 20 μl of distilled water, 20 μl of the sample buffer (0.1 M Tris-HCl/4% SDS/20% glycerol/0.002% bromophenol blue), and 20 μl of the sample buffer containing 1.5% dithiothreitol. Fifteen microliters of the sample was electrophoresed by SDS-polyacrylamide gel electrophoresis on a 3% stacking gel and a 6% separating gel. The proteins separated by electrophoresis were electrotransferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membrane was incubated in blocking solution (2% BSA/2% horse normal serum/2% goat normal serum in PBS) for 1 hour at room temperature. After the membrane was washed with PBS for 5 minutes, it was immunoblotted with the primary antibody (MAb 1G2) at room temperature for 2 hours. The membrane was washed in PBS three times for 5 minutes each, followed by incubation in the secondary antibody for 30 minutes at room temperature. The membrane was again washed with PBS several times, and the immunoblotted protein bands were detected by using chemiluminescence reagent (NEN Life Science Products, Boston, MA) and Hyperfilm ECL (Amersham). The optical densities of immunoreactive bands were measured by a computer-supported analysis, as described above.

**RESULTS**

**RT–PCR Experiments**

RT–PCR using primers specific to neurocan showed that cDNA fragments of the expected length (378 bp) were amplified in experiments using rat retina and brain cDNAs, whereas control PCR experiments without the use of reverse transcriptase did not show any bands (Fig. 1A). Southern blot analysis using an internal oligonucleotide probe was performed in an attempt to confirm the origin of the products amplified by RT–PCR. This showed that the amplified PCR products of the expected length hybridized with internal probes (Fig. 1B), indicating that they were derived from the expected sequence of rat neurocan core protein gene.

**Semiquantitative RT–PCR Experiments**

In an attempt to quantify relative levels of mRNA expression of neurocan core protein gene during retinal development, we carried out semiquantitative RT–PCR experiments and subsequent Southern blot analysis after normalization to β-actin (Fig. 2). The semiquantitative analyses demonstrated that the mean level (±SE) of gene expression for neurocan at embryonal day 16 (E16) was 5.5 ± 1.6-fold that of the adult (P42) retinas (Fig. 3). At early postnatal stages (P0–P3), mRNA expression for neurocan increased gradually, reaching a peak on P7 (8.5 ± 1.9-fold), it then decreased and reached a minimum in adult retinas (P42).

**Immunohistochemical Studies**

The spatial expression of neurocan during retinal development (from E14–P42) was studied by immunohistochemistry using...
an anti-neurocan monoclonal antibody, MAb 1G2. In embryonal stages (E14 and E16), homogeneous retinal (neuroblast) cells were observed throughout the retina (Fig. 4). In early postnatal stages (P0–P7), inner retinal cells became differentiated and formed conspicuous layers consistent with the ganglion cell layer (GCL) and the inner plexiform layer (IPL). The outer retinal layers occurred between P7 and P21.

On E14 and E16, neurocan immunoreactivity was identified throughout the retina (Fig. 4), which was composed of homogeneous retinal cells. In these early embryonal stages, neurocan immunoreactivity was more conspicuous in the inner aspects of retinas than in the outer aspects. Confocal images at high magnification (×400) demonstrated that neurocan immunoreactivity was present primarily in extracellular regions (Fig. 5A). Around birth (E18–P3), as ganglion cells became differentiated, immunohistochemical studies have revealed that pronounced neurocan immunoreactivity is present in inner layers such as the nerve fiber layer (NFL) and the GCL. In adult eyes (P42), S-100β immunoreactivities were detected in GCL (possible astrocytes) and cell bodies in INL (possibly Müller cells). Also, vimentin immunoreactivities were also shown in GCL and radial cells (possibly Müller cells) throughout the retina (Fig. 5B). These immunohistochemical studies showed the usefulness of the antibodies as markers for Müller cells. However, in adult rat retinas, immunohistochemical experiments did not show significant immunoreactivities to neurocan as described above (Fig. 4). On the other hand, on P7 when the expression of neurocan was most conspicuous during the development and when it showed a radial staining pattern in the retina, both S-100β and vimentin immunoreactivities were found only in GCL, and not in the other retinal layers. Confocal microscopic observation of the sections with double staining of neurocan and cell type markers (S-100β and vimentin) revealed no colocalization of the staining patterns at least for retinal Müller cells (Fig. 5C).

Immunoblot Analysis
To characterize the specificity of the anti-neurocan antibody (MAb 1G2) used in this study, we carried out an immunoblot analysis (Fig. 6). Immunoblot analysis using retinal preparations at P0 and P21 and brain preparations at P42, without the use of enzymatic treatment, showed diffuse smear-like bands around 200 to 300 kDa. In immunoblot experiments for proteoglycans, it is well known that diffuse bands are caused by GAG side chains of various sizes bound to a core protein. Thus, in an effort to characterize GAG side chains linked to neurocan core proteins, after the treatment of retinal preparations with protease-free chondroitinase ABC to remove chondroitin sulfate side chains, similar immunoblot experiments were conducted. In both retinal preparations and cerebral samples, immunoblot analysis demonstrated two conspicuous bands. The molecular weights of the two bands were 220 and 150 kDa, which agree with the known molecular weights of neurocan core glycoprotein and its proteolytic C-terminal product.
Temporal Expression of Neurocan Core Protein in the Retina

To elucidate the temporal regulation of neurocan expression in the retina, the retinal samples at various developmental stages from E16 through P42 were treated with chondroitinase ABC and then subjected to immunoblot analysis (Fig. 7A). Immunoblot experiments showed a faint band at 220 kDa but no bands around 150 kDa in retinas on E16. Similar experiments using rat retinas on E18 showed two bands of 220 kDa and 150 kDa. In early postnatal stages (P0–P3), the intensity of each band increased gradually as development proceeded. The intensities of bands of 220 kDa reached a maximum level on P3, whereas those of 150 kDa reached a peak at a later developmental stage (P14). The intensities of both bands decreased after the peaks and reached minimums in adult retinas. In mature retinas on P30 and P42, the bands were barely detectable by immunoblot analysis.

The intensities of immunolabeled bands were semiquantified using a densitometric analysis, and relative levels were calculated as the percentage of the mean levels at the peak (P3 for 220-kDa bands and P14 for 150-kDa bands). The mean level (±SE) of 220-kDa bands on E16 was 15.9% ± 8.1% of the peak intensities on P3 (defined as 100%) and then decreased gradually to its minimum on P42 (17.7% ± 6.3% during the postnatal stages; Fig. 7B). The mean level (±SE) of 150-kDa bands on E16 was 3.9% ± 2.2% of the maximum on P14 (defined as 100%) and then decreased gradually to its minimum on P42, 0.9% ± 0.9% (Fig. 7C). Thus, temporal alterations in protein expression for the 150-kDa band (possible proteolytic C-terminal product of neurocan) were much more distinct than those of the 220-kDa band.

In an effort to calculate proteolytic activities in retinas during development, the relative ratio of the measured optical densities of 150 and 220 kDa bands at each developmental
FIGURE 5. High magnifications (×400) of the immunohistochemical images for neurocan during retinal development (A). On E16, strong immunoreactivity was shown in the extracellular space. Around birth (E18–P3), pronounced neurocan immunoreactivity was present in the inner layers. On P7 and P14, neurocan immunoreactivity appeared to be most conspicuous in the IPL, whereas only moderate neurocan immunoreactivity was seen in the OPL. In the adult retina (B; original magnification, ×200), the anti-S-100β and anti-vimentin antibodies recognized the cell bodies with radial running fibers in the INL and radial cells throughout the retina, respectively (arrows), which indicated the usefulness of the antibodies as markers for Müller cells. Note that these antibody immunoreactivities (Texas red) showed no colocalization with the radial staining patterns (arrowheads) of neurocan immunoreactivities (fluorescein) on P7 (C; magnification, ×400). NBL, neuroblast layer.
DISCUSSION

Neurocan is a nervous tissue-specific proteoglycan and is the major component of CSPG in the brain. It has been shown previously that neurocan regulates neurite outgrowth and cell adhesion in neural tissues. Among a number of proteoglycans, CSPG is regarded as one of the most important neurotrophic factors for retinal neuronal cells, because rat superior colliculus-derived CSPG promotes survival of retinal ganglion cells. It is thus likely that neurocan is a neurotrophic factor for retinal ganglion cells. In previous reports, although immunohistochemical experiments suggested the presence of neurocan in rat retinas, the same group concluded that the immunostaining in retinas is possibly nonspecific because of equally evident staining even with the use of secondary anti-

body alone. Thus, there have been no conclusive data regarding the identification and characterization of neurocan in the retina. In this study, we investigated the expression of a nervous tissue-specific CSPG, neurocan, in developing rat retinas. Our studies demonstrated the expression of mRNAs and core proteins of neurocan in the retina. RT-PCR experiments in this study indicated the presence of mRNA expression of the neurocan gene in the retina as well as in the brain. Subsequent Southern blot analysis using an internal oligonucleotide probe showed that the amplified PCR products were derived from the expected rat neurocan gene sequence. In addition, immunohistochemical studies demonstrated the existence of this core protein in rat retinas, which is consistent with the results from the above-mentioned RT-PCR studies. In rat retinas, our immunohistochemical studies showed neurocan core protein immunoreactivities in the inner retinal layers, and immunoblot analysis demonstrated a diffuse band at approximately 200 to 300 kDa, which agrees with similar experiments using rat brain tissues. It is very likely that the diffuse nature of the immunoreactive band reflects various sizes of GAG moieties linked to the core proteins. This hypothesis is supported by the fact that, after treatment with chondroitinase ABC for the chondroitin sulfate GAG side chains, similar experiments showed two major bands of 220 and 150 kDa, which correspond to the expected molecular weights of neurocan core glycoprotein and its C-terminal proteolytic products, respectively. At present, it has been reported that there are three proteolytic products, 90-kDa and 130-kDa N-terminal fragments and a 150-kDa C-terminal fragment in postnatal rat brain. In this study, we used a monoclonal antibody, MAb 1G2, that can recognize both full-length neurocan and its C-terminal half proteolytic product, as described elsewhere. Thus, we conclude that retinal neurocan immunoreactivities shown by immunohistochemical studies using the MAb 1G2 antibody are indeed derived from the expected proteins of neurocan and its related proteolytic variant. We thus concluded that neurocan (and its proteolytic C-terminal product) is expressed in the rat retina as well as in the rat brain. Our immunohistochemical studies revealed spatial changes in the expression of neurocan core protein in the retina. On E16, when retinal tissues were composed of uniform retinal (neuroblast) cells and when ganglion cells begin to differentiate, neurocan immunoreactivities are homogeneous and relatively faint. Near birth (E18–P3), when ganglion cells and their nerve fibers are undergoing differentiation and maturation, in the innermost layers such as NFL and GCL, the neurocan immunoreactivities are most conspicuous, and then (P7–P42) immunostaining intensities gradually decrease. On P7, when formation of the IPL was observed, the intensities of neurocan immunoreactivities in this layer become quite strong, in contrast to the decrease seen in the NFL and GCL. Similar associations between neurocan immunoreactivities and the formation of a neuronal layer were observed in the developing OPL between P7 and P14. In adult (P42) rat retinas, as all retinal layers complete development, neurocan immunoreactivities decrease throughout the retina. In developing rat retinas, it is well known that the differentiation of retinal cell types and the formation of retinal layers develop gradually from inner to outer layers, even after birth. In general, neurocan has been suggested to be synthesized by neurons. However, an immunoelectronmicroscopic study showed that a 130-kDa proteolytic variant is localized in the
cytoplasm of glial cell processes in the rat cerebrum.²⁷ Our immunohistochemical results revealed the presence of neurocan immunoreactivities in radial cells of P7 rat retinas. Radial staining pattern implies that cell origin for the immunoreactivities may be derived from retinal Müller cells. Thus, in an attempt to investigate this possibility, we carried out further immunohistochemical studies with the use of double-staining techniques using cell type markers (vimentin as well as S-100). However, because of a different time course during the retinal development in the expression of neurocan and cell type markers (vimentin and S-100), we were unable to draw any conclusion on this issue. Further studies will be needed for exact identification of cell origin of neurocan-expressing retinal cells.

On the other hand, neurocan is involved in a number of processes of neural development. For example, cell biological studies showed that neurocan inhibits/promotes neurite outgrowth in a manner dependent on the environment surrounding cultured neural cells.³⁰ Neurocan inhibits neurite-promoting and neural adhesion effects of a number of cell adhesion...
molecules (CAM), such as L1/NgCAM, N-CAM, and tenascin. Balsamo and associates have reported that a chicken retinal CSPG regulates cellular responses to cadherin, another cell adhesion molecule related to neural network formation. In a review article by Margolis et al., this retinal CSPG was hypothesized to be identical to neurocan because they have the same molecular size and amino acid sequence data from CNBr peptides. Also, in cocultures of rat embryonal retina and diencephalon, retinal axons invade the thalamus, although they are repulsed by the hypothalamus under physiological conditions. Immunohistochemical studies revealed that the neurocan staining intensity changes at the border between the thalamus and the hypothalamus, suggesting that neurocan may play a pivotal role in neural networks of the retina. Thus, our immunohistochemical studies, in addition to the above-mentioned findings, suggest that neurocan, its related proteolytic product, or both may regulate neuronal outgrowth and subsequent formation of neural networks in rat retinas during development.

Our molecular biological and biochemical studies showed that the expression of neurocan and its proteolytic variant is regulated in rat retinas temporally as well as spatially. Our semi-quantitative RT–PCR experiments showed that mRNA expression for the neurocan gene is found on E16, that mRNA expression gradually increases during early postnatal stages, and that it reaches its peak between P7 and P14. Then, as retinal differentiation and the formation of neural networks are developed, mRNA expression for neurocan decreases rapidly to the minimum at adult (matured) status on P42. Around P7 and P14, formation of networks between retinal neuronal cells proceeds, especially in the IPL and OPL, which agrees with pronounced neurocan immunoreactivities in these synaptic regions at P7 and P14, as described before. Accordingly, our results suggest that mRNA expression and localization of proteins are closely related to the formation of neural networks in the retina. However, one important point to which we should pay attention is the existence of proteolytic variants of neurocan. In the adult rat brain, neurocan of 220 kDa is cleaved between amino acid residues 638 and 639 by proteolysis and results in the formation of two proteolytic products of 150 and 130 kDa. Although neurocan and its proteolytic C-terminal variant (also called as CSPG-150 or neurocan-C) have similar biological activities, neurocan has been shown to be more effective than the proteolytic C-terminal variant in binding to NgCAM/11. Thus, the proteolytic cleavage of neurocan may play an important role in neural network formation. Because the change from full-length neurocan to proteolytic C-terminal variant is caused by a proteolytic process, our results from RT–PCR experiments on temporally regulated mRNA expression should be regarded as a summation of neurocan and its proteolytic C-terminal variant. Our results demonstrated that the production of neurocan core proteins starts before E16 and reaches its peak at an early postnatal stage (P3). On the other hand, abundant amounts of the proteolytic variant are found transiently in early postnatal stages, then reach a peak at P14 and rapidly diminish in mature stages. Because the predominant variant in the retina is a 150-kDa proteolytic product between P7 and P21, the supposed peak of the summation of both neurocan and its proteolytic C-terminal variant in developing retina is thought to be around P14, which agrees with the possible peak of mRNA expression between P7 and P14. These findings suggest that proteolytic events in the retina are activated around P7 and P14 when mRNA expression reaches its peak and strong neurocan immunoreactivities are shown in synaptic regions. Our results indicate that temporal regulation of the proteolytic processes against neurocan may be different in the retina than in brain because it has been reported that the proteolytic variants are the predominant form in the mammalian retina.

In conclusion, we have demonstrated the expression of mRNAs and core proteins for neurocan (and its proteolytic variant) in the rat retina. Our results show that in developing rat retinas the expression of neurocan is regulated both temporally and spatially, which suggests that it may play an important role in the differentiation of and neural network formation in the mammalian retina.

References

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