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Research report

Postnatal development of NK1, NK2, and NK3 neurokinin receptors expression in the rat retina

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Abstract

The biological effects of tachykinins are mediated by three distinct receptors, the neurokinin 1 receptor (NK1-R), NK2-R, and NK3-R. There is no information available concerning the development of these receptors in the retina. In the present study, we investigated the localization of tachykinin receptors, using antisera directed against NK1-R, NK2-R, and NK3-R in the adult and developing rat retinas. Numerous NK1-R immunoreactive (NK1-R IR) cells were already observed in the proximal part of the neuroblastic layer in the retina at postnatal day 5 (P5). The distribution and intensity of NK1-R IR cells and processes in the inner nuclear layer (INL) and inner plexiform layer (IPL) at P10 were similar to those of adult retina. Most NK1-R IR cells located in the proximal part of INL, which were morphologically amacrine cells. In the contrast to the early expression of NK1-R IR cells, no NK3-R IR structures existed in the neuronal elements of the retina until P10. NK3-R IR processes were first detected in the outer plexiform layer (OPL) at P10. At P15, NK3-R IR somata were slightly stained in the distal and middle parts of the INL, and NK3-R IR processes were present in the OPL and the upper part of the IPL. During P15–P30, the number of NK3-R IR somata located in the INL remarkably increased. These NK3-R IR cells were morphologically bipolar and amacrine cells. This study provides differential cellular distribution of NK1-R IR cells and NK3-R IR cells in the INL of the rat retina. Our findings suggest that NK1-R and NK3-R are involved in different visual circuits and retinal maturation, and NK3-R may play previously unknown important roles in the visual processes of the rat. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Development; Retina; Immunocytochemistry; Tachykinin receptor; Substance P; Amacrine cell; Bipolar cell; Rat

1. Introduction

Tachykinins are a family of biologically active peptides that share a common COOH-terminal amino acid sequence, PHE-X-GLY-LEU-MET-NH_2, and are structurally and functionally related [22,29]. The mammalian tachykinin family is known to be composed of three neuropeptides: Substance P (SP), Neurokinin A (NKA; Substance K, Neurokinin α, Neuromedin L), and Neurokinin B (NKB; Neurokinin β, Neuromedin K). Three peptides are expressed in neurons and are encoded by two genes, one of which produces SP alone or SP and NKA together, the other producing only NKB [25]. They are widely distributed throughout the peripheral and central nervous systems, and their proposed functions include neurotransmitters, modulators, as well as growth factors [2,27,30]. The biological effects of tachykinins are mediated by three distinct receptors, the neurokinin 1 receptor (NK1-R), NK2-R, and NK3-R [29]. Recently, the structures of three receptors have been determined, and they have been found to belong to the class of 7 transmembrane spanning G protein-coupled receptors [20,33,34,44]. All tachykinins can interact with each neurokinin receptor, but with varying affinity (NK1-R: SP > NKA > NKB; NK2-R: NKA > NKB > SP; NK3-R: NKB > NKA > SP) [18,31].

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Table 1
Postnatal days and number of rats used in the present study

Postnatal days	Number
0 D	5
2 D	4
5 D	5
7 D	3
10 D	4
15 D	5
17 D	3
20 D	4
25 D	3
30 D	4
90 D (Adult)	5

The presence of SP has been reported in the amacrine cells and ganglion cells in the adult rat retina using immunohistochemistry, and their dendrites are stratified within sublaminae 1, 3 and 5 of the inner plexiform layer (IPL) [3,5,14,32,35,47]. RNAs demonstrate that SP/NKA encoding transcripts are expressed in the inner nuclear layer (INL), IPL, and ganglion cell layer (GCL), whereas NKB-encoding transcripts are expressed in the GCL [3]. In the receptor binding study, NK1-R, NK2-R, and NK3-R binding sites are distributed in the inner plexiform layer [23]. And NK1-R, NK2-R, and NK3-R mRNAs have been detected in the rat eye by using Northern blots [38]. These studies support the presence of NK1-R, NK2-R and NK3-R in the rat retina.

In the developing study of the rat retina, it was reported that SP containing cells were first detected in the INL by postnatal day 5 (P5), there was a marked increase in the intensity of immunoreactive staining by postnatal day 15 (P15), and the morphology and intensity of SP containing cells attained to adult level at postnatal day 30 (P30) [47]. The findings suggest that tachykinins may be involved in the regulation of retinal maturation and neuronal activity. In the present study, we investigated the cellular localization and developmental period of tachykinin receptors in the rat retina, using antisera directed against NK1-R, NK2-R, and NK3-R, to gain some insights into possible roles played by tachykinins and tachykinin receptors during retinal development and maturation.

2. Materials and methods

2.1. Animals and fixation

Wistar albino rat pups were bred in a laboratory colony from stock originally obtained from Kiwa animal labora-

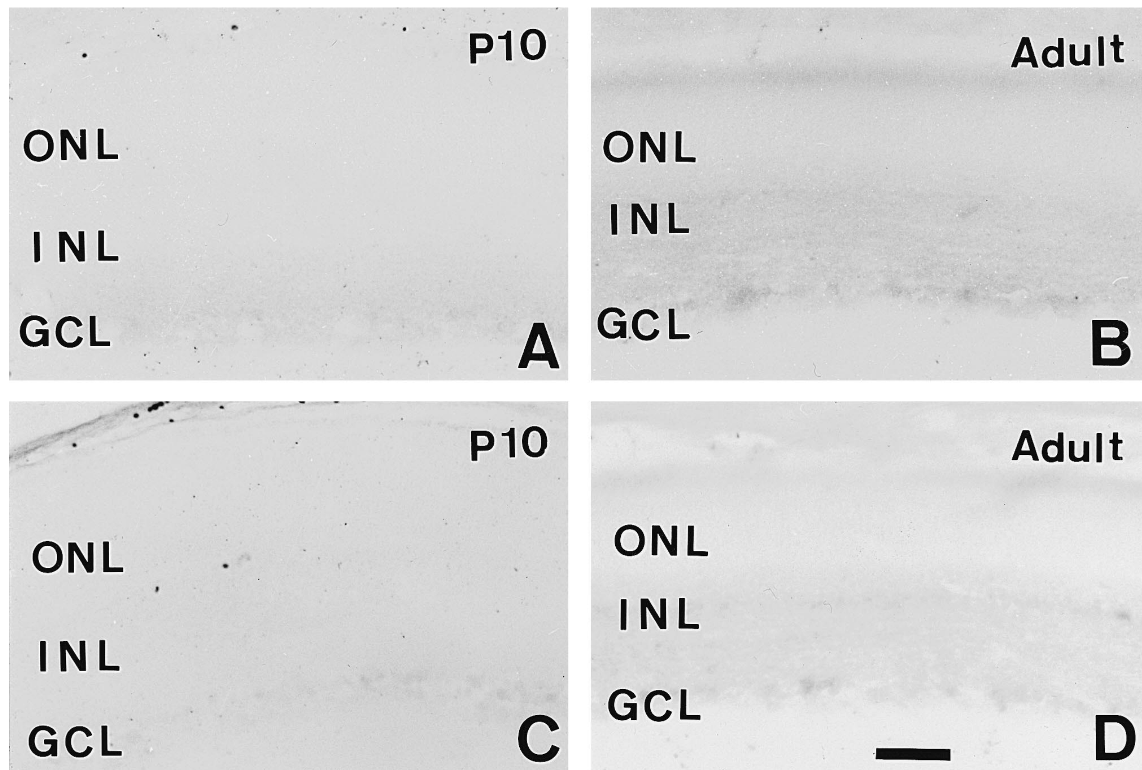

Fig. 1. Photomicrographs indicate that staining was abolished by absorption with the NK1-receptor fragment (A and B) or NK3-receptor fragment (C and D) as described in materials and methods. NK1-R and NK3-R immunoreactive products are not observed in the photoreceptor outer segment, outer nuclear layer (ONL), outer plexiform layer, inner nuclear layer (INL), inner plexiform layer and ganglion cell layer (GCL) at any postnatal stages. A and C, retinas at postnatal day 10 (P10). B and D, retinas at adult stage. Bar, 100 μm.

tory (Wakayama, Japan). The number and postnatal stages of rats used are listed in Table 1. Animals were maintained in a standardized environment with respect to both photoperiods (12-h light/dark; lights on at 08.00 h) with free access to food and water. Lighting was approximately 100 lux. The day of birth was designated as postnatal day 0 (P0).

All animals were anesthetized with sodium pentobarbital (4 mg/100 g, i.p.), and perfused transcardially with 10–50 ml of saline, followed by 2% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (PB, pH 7.4). Eyes were immediately removed, immersed in the same fixative for 24 h at 4°C and rinsed for 48 h at 4°C in 0.1 M PB containing 30% sucrose.

2.2. Immunocytochemistry for NK1-R, NK2-R, NK3-R, and SP

NK1-R, NK2-R, NK3-R, and SP immunoreactive products were visualized by the peroxidase–antiperoxidase (PAP) method. Retinal sections (20 μm thick) were cut perpendicular to the vitreal surface on a cryostat. Sections were detached on PLL coated slide glasses, and stored at −20°C until immunocytochemical staining. Sections were rinsed in 0.1 M phosphate-buffered saline (PBS) at 4°C for 1 h, and incubated in 0.1 M PBS containing 10% normal goat serum (NGS) for 1 h before incubation with the first antisera against NK1-R, NK2-R, NK3-R, or SP. Each antiserum was diluted 1:3000 in 0.1 M PBS containing

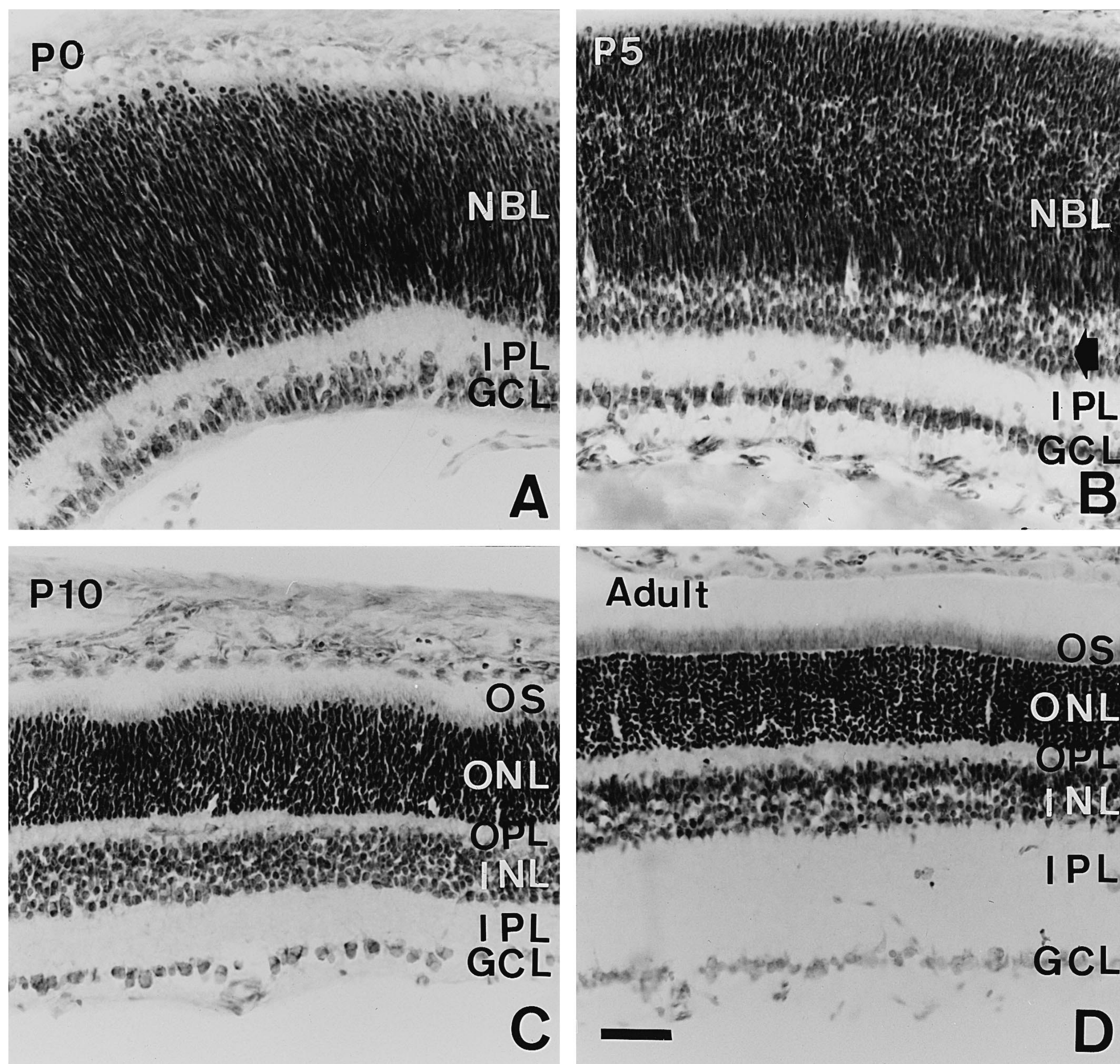

Fig. 2. Nissl-stained retina from birth to adult. (A) The retina at P0 is immature, consisting of a neuroblastic layer (NBL), an inner plexiform layer (IPL), and a ganglion cell layer. (B) At P5, in the proximal part of the NBL (arrow), amacrine cells are visible. (C) At P10, the outer nuclear layer (ONL), outer plexiform layer (OPL), and inner nuclear layer (INL) are clearly divided, and photoreceptor outer segment (OS) can be identified. (D) An adult retina. Bar, 50 μm.

0.3% Triton X-100, 1% NGS and 1% bovine serum albumin. After 72 h of incubation with diluted antiserum at 4°C, sections were rinsed in 0.1 M PBS at 4°C for 1 h and incubated at 4°C for 24 h with a second antiserum, goat anti-rabbit immunoglobulin G (Cappel, 1:1000). Following rinsing as above, the sections were incubated with rabbit PAP complex (Cappel, dilution: 1:1000) at 4°C for 24 h. Peroxidase reaction products were visualized by incubation at room temperature for 20 min in 0.05 M Tris–HCl buffer containing 3,3-diaminobenzidine (20 mg/100 ml), ammonium nickel (II) sulfate hexahydrate (600 mg/100 ml), and 30% hydrogen peroxide (10 μl/100 ml). The sections were then rinsed several times in 0.1 M PBS, dehydrated through a graded series of ethanol and xylene, and cover-slipped in Permount O.

2.3. Antibodies and control experiments

NK1-R antiserum was supplied by Dr. Steven R. Vigna, and NK2-R and NK3-R antisera were provided by Drs. Patrick D. Gamp and Niegel W. Bunnett.

The NK1-R antibody is a rabbit polyclonal antiserum directed to a 15-amino acid peptide sequence [SPR-(393-407)] at the carboxyl terminus of the rat NK1-R [39]. The NK2-R and NK3-R antibodies are rabbit polyclonal antisera against a 15-amino acid peptide sequence [NK2-R(376-390)] and [NK3-R(438-452)] at the carboxyl terminus, respectively [16]. SP antiserum was purchased from Incstar (Lot No. 104560). Several sections at each stage were stained with thionin, in order to investigate the neuronal differentiation of the retina.

The specificities of the antisera were checked with the following experiments: (a) the first antisera omitted, (b) the diluted first antisera were preabsorbed synthesized peptides (10 μM) or receptor fragment (10 μM). When sections were incubated in the preabsorbed NK1-R, NK3-R and SP antisera, specific immunostaining was not observed at any postnatal stages in the retina (Fig. 1). Therefore, immunocytochemically stained structures that were observed in the retina, were considered to be specific for each antiserum. When sections were incubated in the preabsorbed NK2-R antiserum, non-specific immunoreactivity was observed in the photoreceptor outer segment (OS) of the retina at any postnatal stages.

3. Results

3.1. Development of the retina

At P0, the rat retina consists of a neuroblastic layer (NBL), the IPL, and the GCL (Fig. 2A). The retinal structure at P2 appeared similar to that at P0. Although an outer plexiform layer (OPL) was not clearly visible at P5, three sublayers of the NBL could be distinguished. The proximal NBL contained round and pale stained cells (probably amacrine cells), the middle NBL contained elongated and dark-stained cells, and distal NBL contained round and dark-stained cells (Fig. 2B). At P7, there was considerable neuronal differentiation in the NBL. The OPL was clearly distinguishable, whereas the OS was still immature. At P10, the OS was well developed and all layers identified in the adult retina were clearly divided (Fig. 2C,D). Rats usually open their eyes at P15, at which stage the retinal cells are considerably differentiated.

3.2. NK1-R immunoreactivity in the adult and developing retinas

Table 2 shows postnatal developmental pattern of NK1-R, NK2-R and NK3-R immunoreactive (NK1-R IR, NK2-R IR and NK3-R IR) structures in the retina.

The distribution of NK1-R IR structures observed in the adult rat retina is similar to that reported in the previous

Table 2
Postnatal developmental pattern of NK1-R, NK2-R, and NK3-R immunoreactive structures in the retina
−: not detected, +: low, ++: moderate, +++: high.

Postnatal day	PE	OS	ONL (NBL)	OPL (NBL)	INL (NBL)	IPL	GCL
NK1-R IR							
0 D	−	−	−	−	−	+	+
2 D	−	−	−	−	+	+	+
5 D	−	−	−	−	++	++	+
7 D	−	−	−	−	++	++	+
10 D	−	−	−	+	+++	+++	+
15 D	−	−	−	+	+++	+++	+
17 D	−	−	−	+	+++	+++	+
20 D	−	−	−	+	+++	+++	+
25 D	−	−	−	+	+++	+++	+
30 D	−	−	−	+	+++	+++	+
90 D (Adult)	−	−	−	+	+++	+++	+
NK2-R IR							
0 D	−	−	−	−	−	−	−
2 D	−	−	−	−	−	−	−
5 D	−	−	−	−	−	−	−
7 D	−	−	−	−	−	−	−
10 D	−	−	−	−	−	−	−
15 D	−	−	−	−	−	−	−
17 D	−	−	−	−	−	−	−
20 D	−	−	−	−	−	−	−
25 D	−	−	−	−	−	−	−
30 D	−	−	−	−	−	−	−
90 D (Adult)	−	−	−	−	−	−	−
NK3-R IR							
0 D	−	−	−	−	−	−	−
2 D	−	−	−	−	−	−	−
5 D	−	−	−	−	−	−	−
7 D	−	−	−	−	−	−	−
10 D	−	+	−	+	−	−	−
15 D	−	+	−	+	+	+	−
17 D	−	+	−	++	++	++	−
20 D	−	+	−	++	++	++	−
25 D	−	+	−	++	++	++	−
30 D	−	+	−	++	++	++	−
90 D (Adult)	−	+	−	++	++	++	−

investigation [6]. The majority of NK1-R IR cells were existed in the proximal part of the INL and they were localized to amacrine cells (Fig. 3A). These cells gave rise to a single process that descended toward the IPL and ramified in the IPL (Fig. 3B). NK1-R IR processes were densely distributed in all IPL sublaminae. A few NK1-R IR processes with somata located in the INL ramified in the OPL. Some NK1-R IR cell bodies were observed in the GCL, which cells gave rise to processes that arborized in the IPL (Fig. 3C).

At P0, some NK1-R IR deposits were observed in the IPL adjacent to the NBL (Fig. 4A). At P2, some weakly stained NK1-R IR somata were existed in the proximal part of the NBL and NK1-R IR processes were observed in

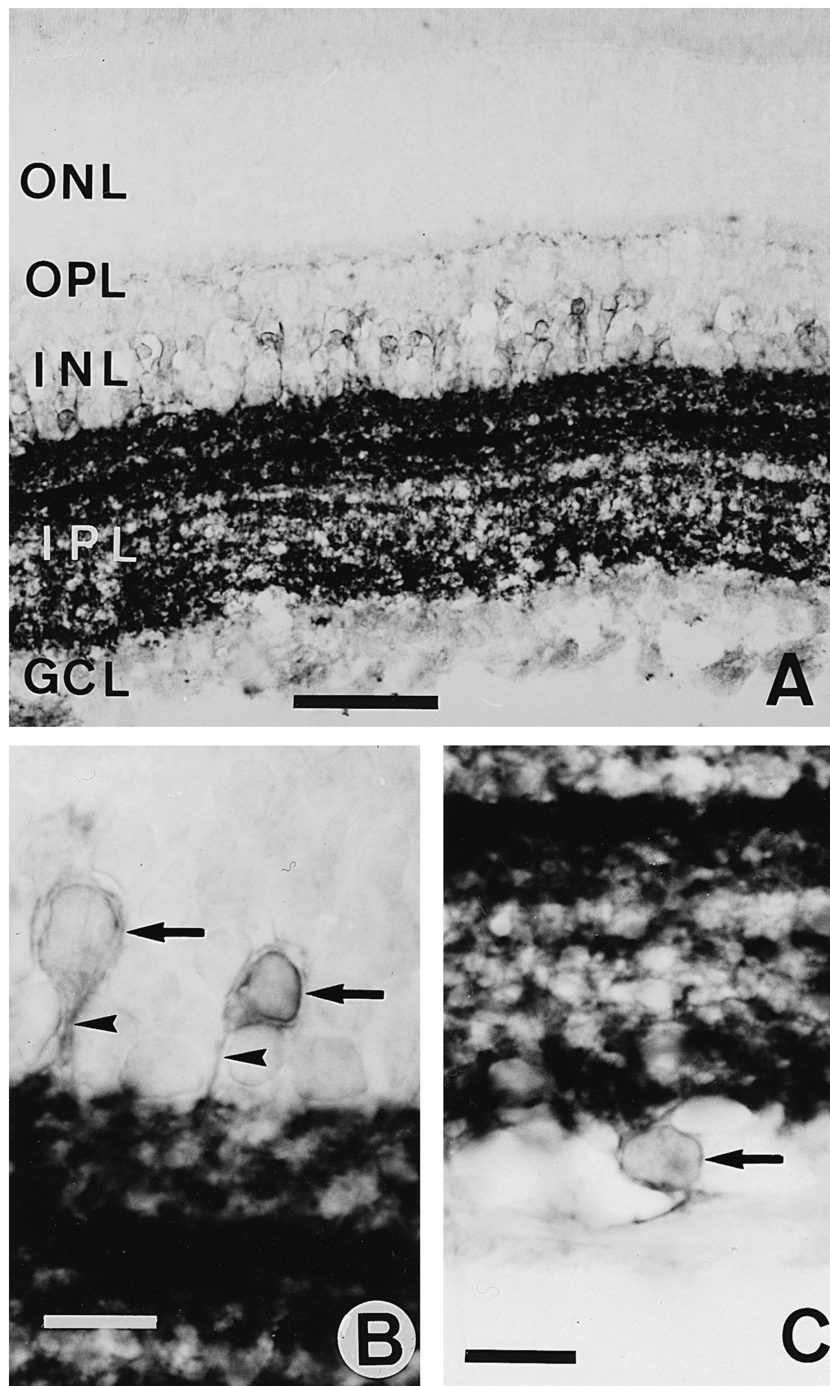

Fig. 3. Neurokinin 1-receptor immunoreactive (NK1-R IR) cells and processes in the adult retina. (A) Numerous NK1-R IR cells are present in the proximal part of the inner nuclear layer (INL) and densely stained NK1-R IR processes are evident in the inner plexiform layer (IPL). NK1-R IR plexus is seen in the outer plexiform layer (OPL). GCL, ganglion cell layer. ONL, outer nuclear layer. Bar, 50 μm. (B,C) NK1-R IR somata (arrows) exist in the inner nuclear layer (B) and ganglion cell layer (C). Arrowheads show descending NK1-R IR processes. Bar, 10 μm.

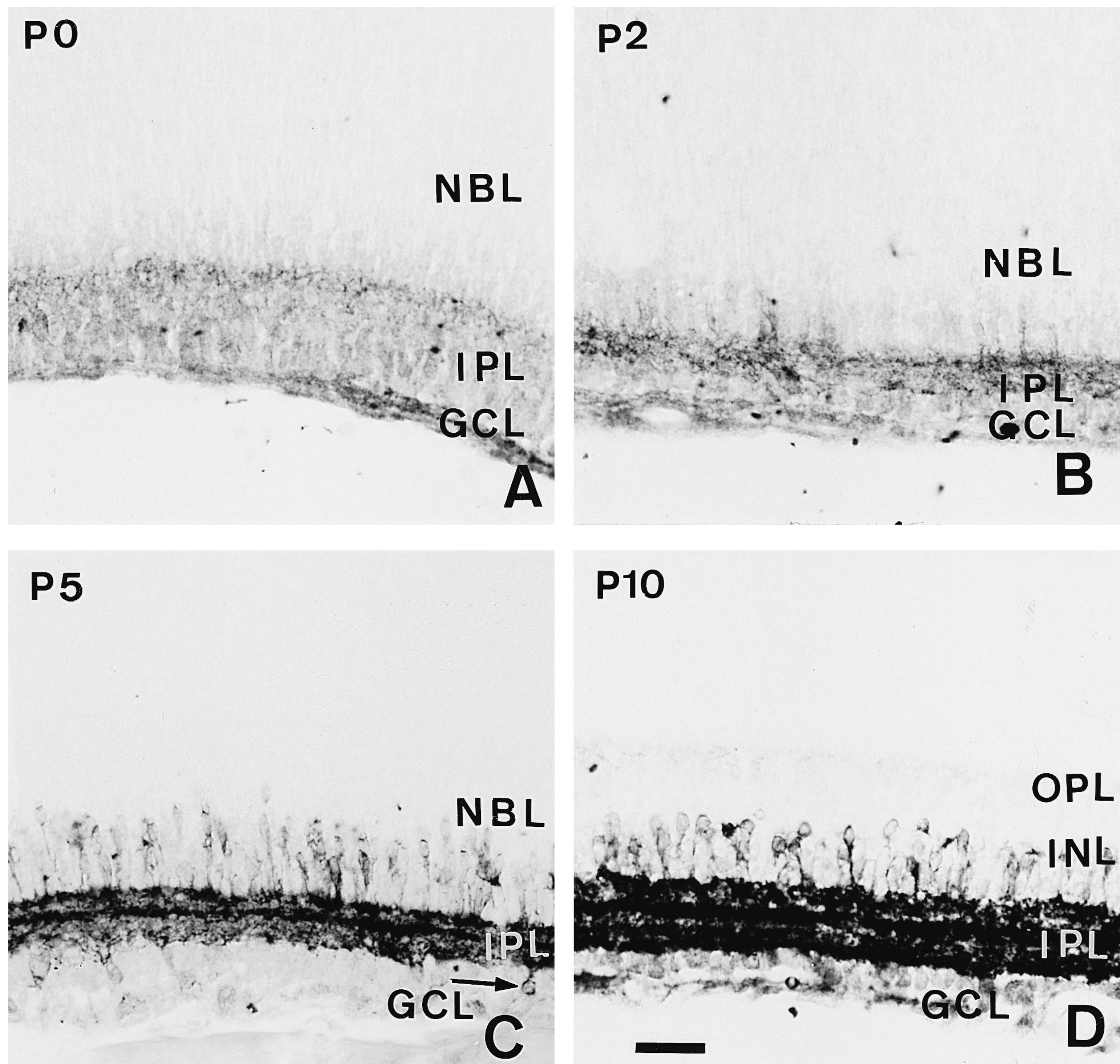

Fig. 4. Expression of neurokinin 1-receptor immunoreactive (NK1-R IR) cells and processes in the developing retina. (A) At P0, a few NK1-R IR deposits are present in the ganglion cell layer (GCL) and inner plexiform layer (IPL) adjacent to the neuroblastic layer (NBL). (B) At P2, some weakly stained NK1-R IR somata with descending process are observed in the proximal part of the NBL. (C) At P5, numerous NK1-R IR neurons are present in the proximal part of the NBL and moderately stained NK1-R IR processes are evident in the IPL. NK1-R IR neuron (arrow) exists in the GCL. (D) At P10, NK1-R IR somata exist in the proximal part of the INL and NK1-R IR processes are stained more stronger than those at P5. NK1-R IR plexus is slightly seen in the outer plexiform layer (OPL). Bar, 50 μm.

the upper part of the IPL (Fig. 4B). Although the NBL was not clearly differentiated into the INL and ONL at P5, numerous NK1-R IR cells were observed throughout the proximal part of the NBL. There was no NK1-R IR structures in the distal part of the NBL (Fig. 4C). NK1-R IR somata were preferentially small in size and elongated in shape. These somata gave rise to a single stout process which descended to and ramified within all sublaminae of the IPL. The NK1-R IR cells were morphologically amacrine cells. At this stage, there were some NK1-R IR cells in the GCL. These cells were small to medium size and gave rise to several processes that ramified within the IPL. The distribution and staining intensity of NK1-R IR cells and processes in the NBL and IPL at P7 were similar to those at P5.

Although the distribution pattern of NK1-R IR cells and processes in the retina at P10 was similar to that at P5 and P7, the intensity of immunoreactivity at P10 was stronger than that at P5 and P7 (Fig. 4D). At P10, NK1-R IR cells were also present in the proximal part of the INL and GCL, whose processes were densely distributed in all IPL sublaminae. A few NK1-R IR somata were observed in the distal and middle parts of the INL, and several NK1-R IR processes formed a sparse plexus in the OPL. The distribu-

tion and staining intensity of NK1-R IR cells and processes at P10 were similar to those at P15, P20, P25, P30, and adult.

3.3. NK2-R immunoreactivity in the adult and developing retinas

Although no NK2-R IR structures were observed in the neuronal elements of the rat retina during postnatal stages, non-specific NK2-R IR structures were observed in the OS of the adult and developing retinas.

3.4. NK3-R immunoreactivity in the adult and developing retinas

In the adult retina, NK3-R IR cells were observed in the INL. The majority of NK3-R IR somata located in the distal and middle parts of the INL and many NK3-R IR

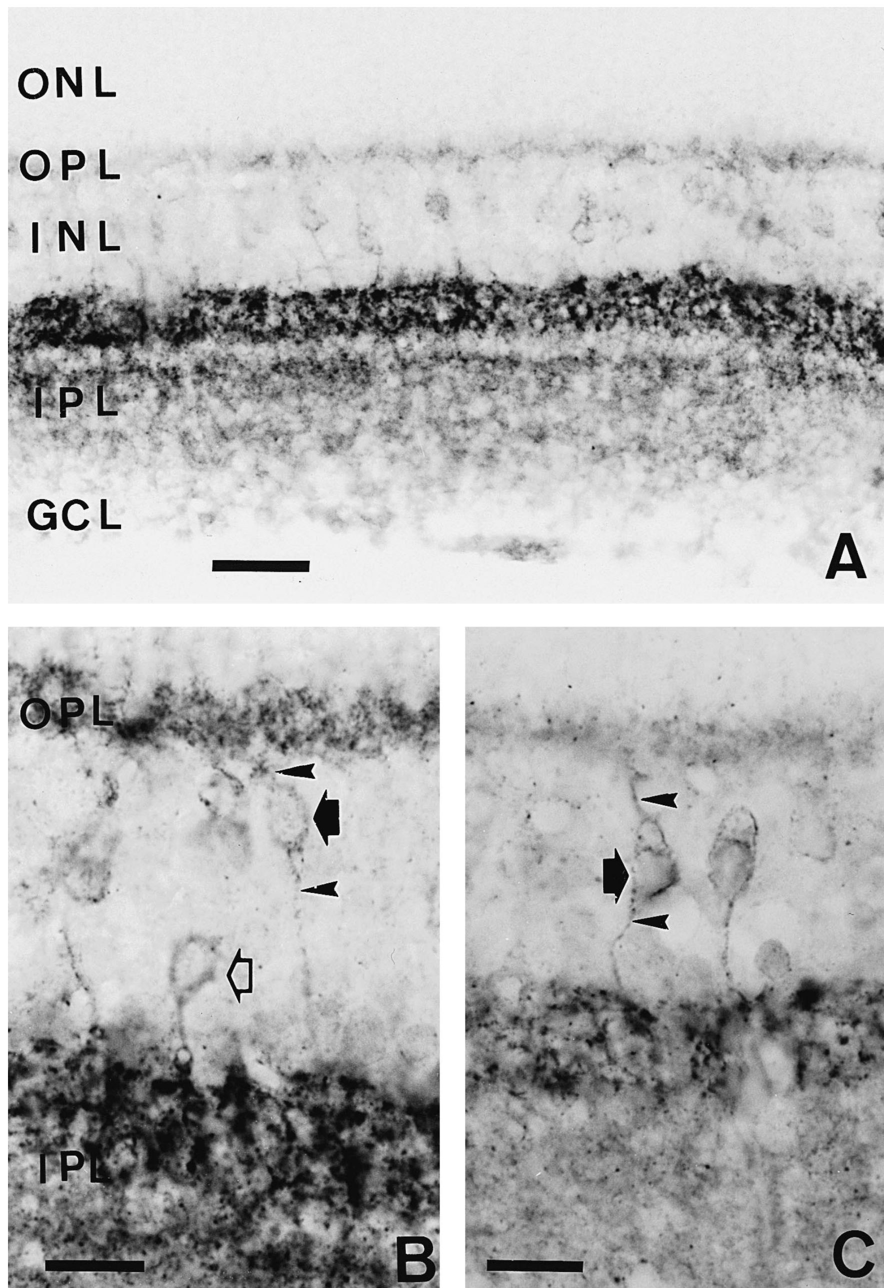

Fig. 5. Neurokinin 3-receptor immunoreactive (NK3-R IR) cells and processes in the adult retina. (A) Many NK3-R IR somata are present in the distal and middle parts of the inner nuclear layer (INL) and a few NK3-R IR cells exist in the proximal part of the INL. Densely stained NK3-R IR processes are evident upper part of the inner plexiform layer (IPL). Moderately stained NK3-R IR plexus is seen in the outer plexiform layer (OPL). GCL, ganglion cell layer. ONL, outer nuclear layer. Bar, 50 μm. (B,C) NK3-R IR neurons (arrows) exist in INL. Solid arrows show bipolar cells with ascending and descending processes (arrowheads) and open arrow indicates amacrine cell. Fig. 4B is the enlargement in D at P30. Bar, 10 μm.

processes existed in the upper part of the IPL and OPL (Fig. 5A). Some NK3-R IR somata were piriform in shape (Fig. 5B,C). The NK3-R IR cells were morphologically divided into two cell types. One cell type was mainly located in the distal and middle part of the INL. This cell type gave rise to a process which ascended to and ramified within the OPL, while an axon-like process descended to and ramified in the upper part of the IPL. This type of NK3-R IR cells resembled bipolar cells based on their position in the INL and their stratification, which were illustrated by Cajal [4]. The second cell type was mainly located in the middle part of the INL, and a single process descended in upper part of the IPL. The second type of NK3-R IR cells was morphologically similar to amacrine cells. NK3-R IR cells and processes were not observed in the GCL and nerve fiber layer in the adult retina.

In contrast to the developmental expression of NK1-R IR, no NK3-R IR structures existed in the neuronal elements of the retina until P10. At P10, NK3-R IR processes appeared in the OPL, whereas NK3-R IR cells were not detected in the INL (Fig. 6A). At P15, immunoreactive fiber plexus was detected in the OPL and IPL adjacent to

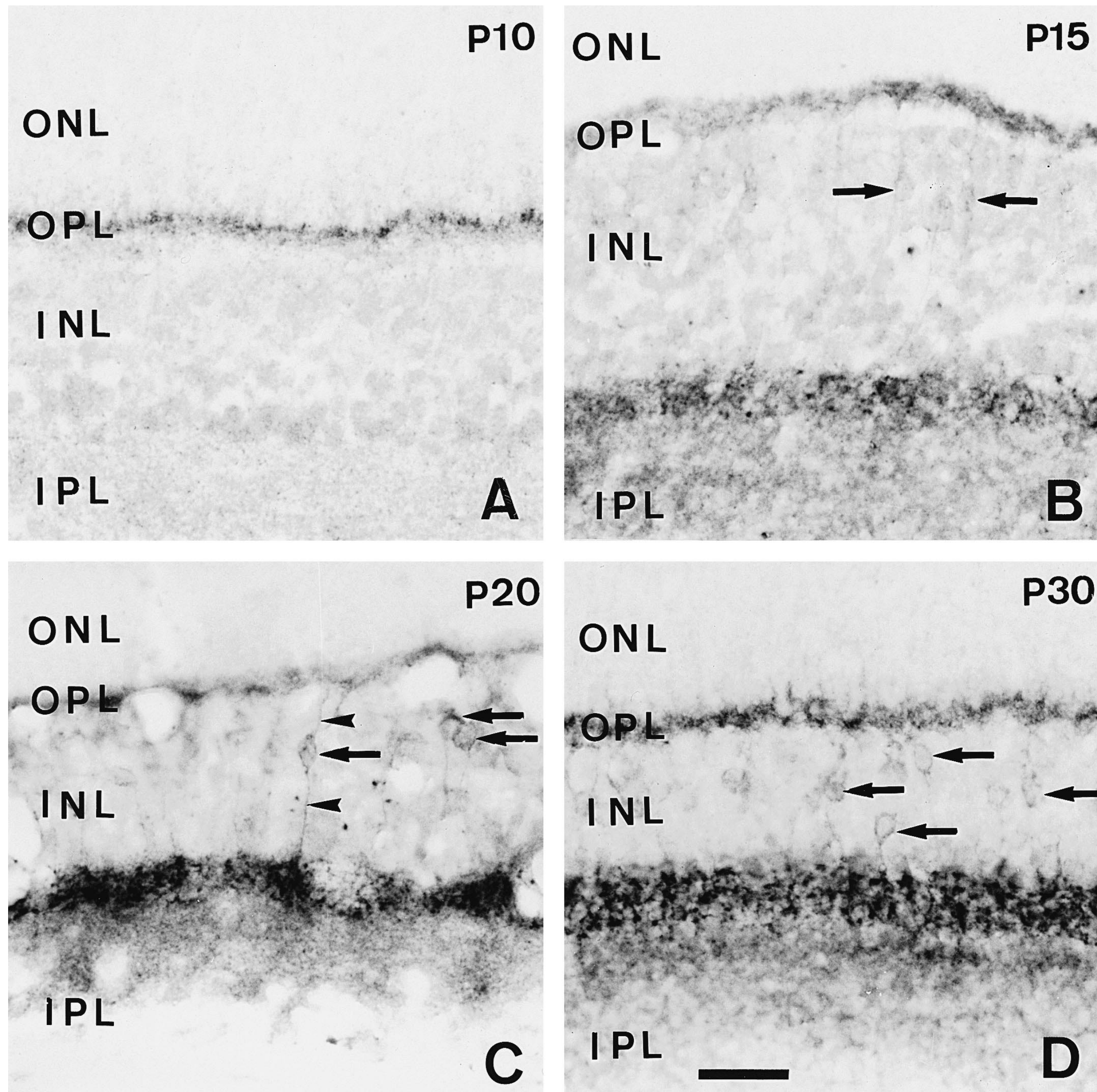

Fig. 6. Expression of neurokinin 3-receptor immunoreactive (NK3-R IR) cells and processes in the developing retina. (A) At P10, there are NK3-R IR deposits in the outer plexiform layer (OPL) whereas there are no NK3-R IR products in other retinal layers. (B) Weakly stained NK3-R IR bipolar cell bodies (arrows) exist in the inner nuclear layer (INL) at P15. Ascending and descending processes ramify in the outer plexiform layer (OPL) and in the upper part of inner plexiform layer (IPL). (C) Some NK3-R IR bipolar cells (arrows) exist in the distal part of the INL. Arrowheads show descending and ascending processes. (D) Many NK3-R IR bipolar and amacrine cells are seen in the distal and middle parts of the INL at P30. Bar, 50 μm.

the INL. Some NK3-R IR cells in the distal part of the INL were weakly stained (Fig. 6B). During P15–P30, the number of NK3-R IR cells gradually increased in the INL (Fig. 6B–D). The morphological features, the distribution, and staining intensity of NK3-R IR cells and processes at P30 were similar to those of the adult retina.

3.5. SP immunoreactivity in the adult and developing retinas

In the present study, we investigated SP immunoreactive (SP IR) structures in the developing retina, in order to compare with the development of the NK1-R IR, NK2-R IR and NK3-R IR cells, and to demonstrate the co-expression of a tachykinin and its receptor in the retina. The distribution of SP IR structures observed in the adult rat retina is similar to that reported in the previous investigations [3,5,14,35,47]. SP IR somata were located in the INL adjacent to the IPL and GCL, and SP IR processes existed in the sublaminae 1, 3 and 5 of the IPL (Fig. 7A).

There were no SP IR structures in the retina until P5. At P5, a few SP IR cells first appeared in the proximal part of the NBL and GCL. The SP IR cells were round or piriform-shaped. SP IR processes existed in the sublamina 5 of the IPL, just above the GCL. At P10 and P15, SP IR cells existed in the most proximal part of the INL and GCL, and SP IR processes were observed in the sublaminae 1 and 5 of the IPL (Fig. 7B). Although the distribution of SP IR cells in the retina at P20 was similar to that at P10 and P15, weakly stained SP IR processes were observed in the sublamina 3 of the IPL, in addition to the sublaminae 1 and 5. The number of SP IR cells in the INL was slightly increased until P30. At P30, the distribution pattern and staining intensity of SP IR cells in the retina were similar to those of adult retina.

4. Discussion

The present study revealed the expression of NK1-R IR and NK3-R IR cells and processes in the adult and developing rat retinas. A significant finding of this study is that the expression of NK1-R IR cells in the INL is already established before eye opening, whereas the expression of NK3-R IR cells in the INL gradually increases after eye open. In addition, the developmental expression pattern of SP IR cells is similar to that of NK3-R IR cells rather than that of NK1-R IR cells.

It has been previously reported that SP IR amacrine cells do not show NK1-R immunoreactivity [6]. In the present study, NK3-R IR cells existed in the distal and middle parts of the INL, while SP IR cells were observed in the most proximal part of the INL adjacent to the IPL. Therefore, NK3-R IR cells is probably different with SP IR amacrine cells in the INL.

4.1. Development of the SP

In agreement with previous studies [14,32,47], we confirmed the following: SP IR cells first appear in the NBL and GCL at P5, their cell number gradually increases in the INL adjacent to the IPL from P5 to P15, and a progressive increase in SP IR cells continues until P30. Developmental studies of neurotransmitter or neuropeptides containing amacrine cells and bipolar cells in the rat retina suggest that some neurotransmitters or neuropeptides may play important roles in the retinal maturation and neuronal

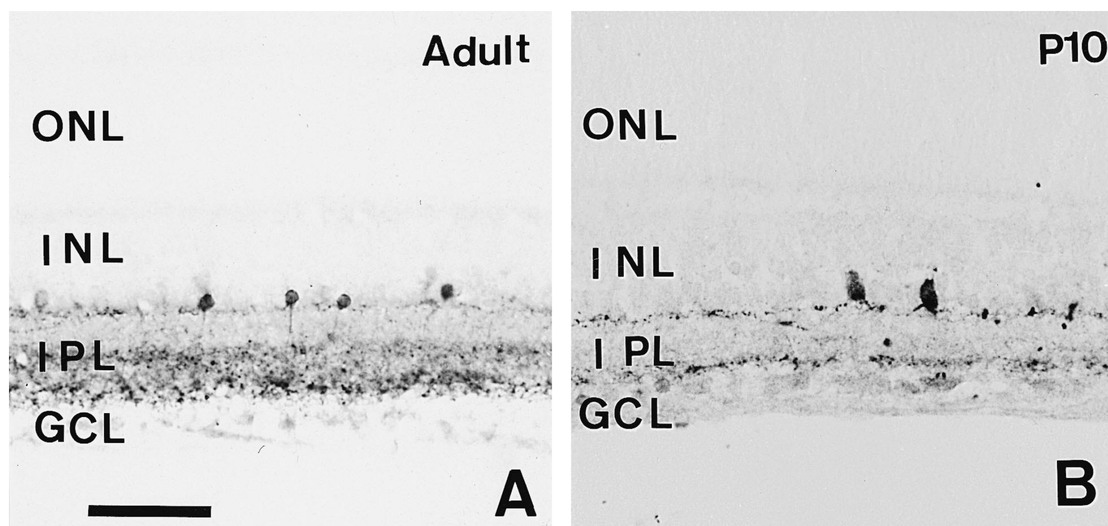

Fig. 7. Substance P immunoreactive (SP IR) cells and processes in the adult retina (A) and retina at P10 (B). Some SP IR amacrine cells exist in the most proximal part of the inner nuclear layer (IPL) and SP IR processes distribute in the sublaminae 1, 3, and 5 of the inner plexiform layer (IPL). At P10, SP IR cells exist in the most proximal part of the INL and SP IR processes are present in the sublaminae 1 and 5 of the IPL. GCL, ganglion cell layer. ONL, outer nuclear layer. Bar, 50 μm.

activity. For instance, dopamine, corticotropin-releasing factor, γ-aminobutyric acid (GABA), and glycine are synthesized in amacrine and bipolar cells in the rat retina during 1–2 weeks after birth [13,21,46]. Furthermore, it was reported that GABA reaches adult expression level at P7 and glycine takes 11 days, whereas glutamate within amacrine cells takes 23 days to reach at adult expression level [13]. The differential expression of these cells indicates that they may have a specific function during retinal development. In the rat retina, retinal neuroblastic cells start to divide on the 5th day, and their development is nearly completed by the 12th day [41,42]. On the 12th day, synapses are formed in the IPL and the first responses have been recorded on the electroretinogram [41]. In the adult retina, SP plays modulatory roles on the electric activity and transmitter release in the retinal neurons [1,45]. Therefore, SP may play an important role in retinal maturation and neuronal pathways during P5–P30.

4.2. Differential development of the NK1-R, NK2-R, and NK3-R

No information is currently available on the timing of the appearance of NK1-R IR, NK2-R IR, and NK3-R IR cells in developing rat retina. In the present study, NK1-R IR cells first appeared in the NBL and GCL at P2 and their cell number gradually increased in the proximal part of the NBL until P10, at which their expression level appeared morphologically mature. Meanwhile, NK3-R IR cells were first observed in distal and middle parts of the INL at P15 and their expression level appeared morphologically mature at P30. Rat retina morphologically undergoes considerable development within the first 2 weeks after birth [13,41,42]. Our results show that the developmental expression of NK1-R IR cells and processes precede the appearance of SP IR cells and the establishment of synaptogenesis in the IPL, while the expression of NK3-R IR neurons appear after the establishment of synaptogenesis. Neurotransmitters or neuropeptides, their receptors, and high affinity uptake systems often develop prior to the onset of synaptogenesis, suggesting that these substances are involved in more than neurotransmission during development [7]. Therefore, our findings suggest that NK1-R IR neurons may relate to the retinal development and NK3-R IR neurons may involve the modulation of the visual transmission in the IPL and OPL levels.

In the present study, we detected differential cellular distribution of NK1-R IR cells and NK3-R IR cells in the INL. Most NK1-R IR cells located in the proximal part of the INL, which were morphologically amacrine cells. On the other hand, most NK3-R IR cells located in the distal and middle parts of the INL, which were morphologically bipolar and amacrine cells. These findings suggest that NK1-R IR cells and NK3-R IR cells in the INL belong to different cell populations, although the possibility may exist that NK1-R and NK3-R co-localize in a few amacrine cells in the INL.

4.3. Function of tachykinin receptors

This study provides evidence for the existence of a distinct distribution pattern of cells and processes with NK1-R IR and NK3-R IR in the INL and IPL. Our findings suggest that NK1-R and NK3-R may be involved in different functional circuits in the retina. In the gold fish retina, SP IR processes make synapses on dendrites of amacrine and ganglion cells and receive synaptic input from these cells [43]. Exogenously applied SP depolarizes amacrine cells and has a long lasting excitatory effect on the majority of ganglion cells [9,10,15,45]. It was reported that NK1-R IR is not present in SP IR amacrine cells and 91% of NK1-R IR cells show GABA immunoreactivity [6]. Therefore, it appears that SP is released from amacrine cells and act on NK1-R IR amacrine cells containing GABA.

In the present study, we found that NK3-R IR cells are morphologically bipolar cells whose axons arborize in the distal part of the IPL. Visual information is segregated into parallel ON and OFF pathways at the level of retinal bipolar cells. Two types of bipolar cells are found in mammalian retina: rod bipolar cells and cone bipolar cells. Both have characteristic dendritic trees in the OPL where they receive input signals from rods and cones, respectively, and their axons in the IPL that provide output on AII amacrine or ganglion cells. It was previously supported that in rod-dominated retinas, such as that of cats and rats, the great majority of all bipolar cells are rod bipolar cells, and all rod bipolar cells seem to be of ON type, and light causes depolarization of rod bipolar cells in the retina [8,40]. Protein kinase C (PKC) immunoreactivity is a reliable marker for rod bipolar cells and PKC immunoreactive cell bodies are usually found in the distal part of the INL, and their axons descended towards the proximal border of the IPL close to the GCL [17,26,28]. PKC immunoreactive sites simultaneously contain mGluR6 protein at the bipolar postsynaptic site [28]. The retinal bipolar cells receiving glutamate transmission from photoreceptors mediate in segregating visual signals into ON center and OFF center pathways. Since knockout mice lacking mGluR6 show a loss of ON responses but unchanged OFF responses to light, it appears that mGluR6 is essential for synaptic transmission to ON bipolar cells [24].

On the other hand, Cajal classified several bipolar cell types, including bipolar cells that arborize in the more distal part of the IPL (d and e type bipolar cells in Fig. 4 of Plate V in Cajal: The Structure of the Retina [4]). Recently, it has been reported that although the rat retina contains 99% rods, at least 50% of all bipolar cells are composed of cone bipolar cells which are divided into 9 different subtypes in the rat retina [12,19]. According to the position of NK3-R IR somata in the INL, and the branching pattern and stratification level of NK3-R IR axon terminals in the IPL, NK3-R IR cells may correspond to types 1–4 of cone bipolar cells described by Euler and

Wassle [12] and Hartveit [19]. In situ hybridization histochemistry demonstrated NKB-encoding mRNAs are expressed only in small-to-medium somata located in the GCL [3]. Although information assessing NKB effects on retinal function is lacking, the present study suggests that a feedback pathway may exist between NKB containing ganglion cells and NK3-R containing cone bipolar cells. Immuno-electron-microscope and PKC immunocytochemical studies will be needed to determine whether or not NK3-R IR cells are cone bipolar cells. The present study has demonstrated that NKB and its receptor may play previously unknown but more important roles in the visual processes of the rat retina.

4.4. Mismatch of tachykinin peptides and their receptors

Recently, immunocytochemistry for NK1-R, NK2-R, and NK3-R using specific antibodies has allowed the identification of specific target cells for SP, NKA, and NKB actions in the retina and gastrointestinal tract of the rat [6,16,39]. Tachykinins are widely distributed in the central and peripheral nervous systems and act via NK1-R, NK2-R, and NK3-R [22]. Immunocytochemical studies have demonstrated that some amacrine and ganglion cells contain SP, and SP immunoreactive processes are present in sublaminae 1, 3 and 5 of the IPL in the rat retina [3,5,14,32,35,47]. Mantyh et al. reported tachykinin receptor specific binding sites for SP, substance K, and neuromedin K are present over the IPL in the adult retina [23]. However, they could not detect the binding sites in cells of the INL and GCL. Furthermore, Tsuchida et al. investigated relative levels of three tachykinin receptor mRNAs in the adult rat central and peripheral tissues [38]. They demonstrated that three tachykinin receptor mRNAs exist in the eye and signals for neuromedin K receptor mRNA (NK3-R) are most prominent among three tachykinin receptor mRNAs [38]. Although they could not determine whether for neuromedin K receptor mRNA expression occurs in the neuronal or non-neuronal portions of the eye, we have confirmed the existence of NK3-R IR neurons in the INL in the present study.

Recently, Casini et al. [6] reported that NK1-R IR cells are present in the proximal part of the INL and GCL of the adult rat retina and these cells are amacrine cells and displaced amacrine cells. In the present study, we have confirmed the expression of NK1-R IR cells and processes in the adult retina. Casini et al. [6] previously suggested that there is a significant mismatch between the localization of NK1-R IR processes and SP processes in the rat retina. There are many examples of mismatches between the distribution of neurotransmitters and their receptors in the central and peripheral nervous systems [36,37], suggesting that neurotransmitters can act in a paracrine fashion. Duggan et al. [11]colleagues demonstrated that primary afferent-derived NKA can diffuse several millimeters from its release site in the substantia gelatinosa of the spinal cord. Although SP displays highest affinity for NK1 receptor, whereas NKA and NKB bind preferentially to NK2 and NK3 receptors, respectively, the selectivity of these peptides for their preferred receptors is poor. Therefore, it is likely that their physiological actions are mediated via their interaction with all three receptors [18,31].

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