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REGULAR ARTICLE

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Light- and electron-microscopic analysis of neuropeptide Y-immunoreactive amacrine cells in the guinea pig retina

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Abstract We investigated the morphology and synaptic connections of neuropeptide Y (NPY)-containing neurons in the guinea pig retina by immunocytochemistry, using antisera against NPY. Specific NPY immunoreactivity was localized to a population of wide-field and regularly spaced amacrine cells with processes ramifying mainly in stratum 1 of the inner plexiform layer (IPL). Double-label immunohistochemistry demonstrated that all NPY-immunoreactive cells possessed glutamic acid decarboxylase 65 immunoreactivity. The synaptic connectivity of NPY-immunoreactive amacrine cells was identified in the IPL by electron microscopy. The NPY-labeled amacrine cell processes received synaptic input from other amacrine cell processes and bipolar cell axon terminals in stratum 1 of the IPL. The most frequent postsynaptic targets of NPY-immunoreactive amacrine cells were other amacrine cell processes. Synaptic outputs to bipolar cells were also observed in a small number of cases. This finding suggests that NPY-containing amacrine cells may influence inner retinal circuitry in stratum 1 of the IPL, thus mediating visual processing.

Keywords Neuropeptide Y · Immunocytochemistry · Synaptic connectivity · Retina · Guinea pig

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Introduction

Neuropeptide Y (NPY), originally isolated from porcine brain (Tatemoto 1982; Tatemoto et al. 1982), is a 36-amino-acid peptide that is known to have multiple and diverse physiological actions in both the central and peripheral nervous system (Lundberg et al. 1996; Munglani et al. 1996; Balasubramaniam 1997; Blomqvist and Herzog 1997; Sun et al. 2001). NPY is present and abundant in both the peripheral and central nervous system, including the retina (Adrian et al. 1983; Brunn et al. 1984).

Immunocytochemical studies have demonstrated the presence of NPY immunoreactivity in the retina of several mammals, including man, monkey, cat, guinea pig and rat (Bruun et al. 1984, 1991; Marshak et al. 1986; Tornqvist and Ehinger 1988; Ferriero and Sagar 1989; Li and Lam 1990; Hutsler et al. 1993; Jen et al. 1994; Jotwani et al. 1994; Hutsler and Chalupa 1994, 1995: Oh et al. 1999). In most mammalian retinas, NPY is localized predominantly to sparsely occurring amacrine cells, some displaced amacrine cells with widely ramifying processes in distinct strata of the inner plexiform layer (IPL). In addition, small ganglion cells showing NPY immunoreactivity have been reported in the cat retina (Hutsler et al. 1993; Hutsler and Chalupa 1994). The NPY gene is expressed by cells in both the inner nuclear layer (INL) and ganglion cell layer (GCL) in the mouse retina (Ammar et al. 1998).

A functional role of NPY has been suggested for the mammalian retina; NPY has been shown to modulate neuronal activity by inhibiting intracellular cyclic adenosine monophosphate (cAMP) concentrations in the rabbit retina (Bruun et al. 1984). Moreover, endogenously applied NPY stimulates the release of glycine, dopamine and 5-hydroxytryptamine in the rabbit retina (Bruun and Ehinger 1993). In addition, functional NPY receptors have been shown to be present in the rat retina (Ammar et al. 1998). Taken together, these anatomical and physiological studies indicate that NPY may act as a neuromodulator in the mammalian retina.

We conducted the present study on the guinea pig retina as part of our continuing efforts to investigate the morphology, topographic distribution, and synaptic organization of peptidergic neurons in the rodent retina. We report that NPY-immunoreactive neurons consist of a population of amacrine cells with processes ramifying mainly in stratum 1 of the IPL, and that they comprise a subpopulation of GABA-immunoreactive amacrine cells. Furthermore, we define the synaptic connectivity of these NPY-immunoreactive amacrine cells.

Materials and methods

Tissue preparation

Five adult guinea pigs of either sex were used. The animals were treated according to the regulations of the Catholic Ethics Committee of the Catholic University of Korea, Seoul, which conform to all the National Institute of Health (NIH) guidelines. The animals were deeply anesthetized by intraperitoneal injection of 4% chloral hydrate (1 ml/100 g body weight). The eyes were enucleated, and the animals were killed by an overdose of 4% chloral hydrate. The anterior segments of the eyes were then removed, and the eyecups were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 2–3 h. Following fixation, the retinas were carefully dissected and transferred to 30% sucrose in PB for 24 h at 4°C. They were then frozen in liquid nitrogen, thawed, and rinsed in 0.01 M phosphate-buffered saline (PBS; pH 7.4).

Immunohistochemistry

Immunostaining was performed using the avidin-biotin-peroxidase complex (ABC) method (Hsu et al. 1981). Whole-mount preparations and 40-µm-thick vibratome sections were incubated in 10% normal goat serum (NGS) and 1% Triton X-100 in PBS, for 1 h at room temperature, to block nonspecific binding sites. These were then incubated with an affinity purified rabbit polyclonal antibody directed against NPY (AB 8711; diluted at 1:1000) in PBS containing 0.5% Triton X-100 for 3 days at 4°C. This antibody was produced by the Antibody/RIA Core of the CURE/Digestive Diseases Research Center and generously provided by H. Wong and Dr. J.H. Walsh of the University of California at Los Angeles. Specificity of immunostaining was evaluated by preadsorbing the antibody with 10^{-6} M NPY (Bachem, Torrance, CA) overnight at 4°C and by using it in place of the primary antibody solution during the overnight incubation. Retinas were washed in PBS for 45 min (3×15 min), incubated for 12 h in biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) with 0.5% Triton X-100 at 4°C, rinsed in PBS, and subsequently incubated in ABC (Vector Laboratories) in PBS for 12 h at 4°C. Retinas were rinsed in two changes of PBS and three changes of 0.05 M TRIS-HCl buffer (TB), pH 7.6, for 5 min each at room temperature, and then incubated in 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) in TB for 10 min. Hydrogen peroxide was added to the incubation medium to a final concentration of 0.01%. The container was gently shaken as the reaction proceeded. The reaction was stopped with several washes of TB and PB after 1–2 min, as determined by the degree of staining. The retinas were mounted on gelatin-coated slides with the ganglion cell layer (GCL) facing upwards, and coverslips were applied with glycerol.

For double-label studies, sections were incubated overnight in a mixture of anti-NPY antibody (1:1000) and mouse monoclonal anti-GAD65 antibody (Boehringer-Mannheim, Germany) with 0.5% Triton X-100 in 0.1 M PB at 4°C. Sections were rinsed for 30 min with 0.1 M PBS, and incubated in Cy3-conjugated goat anti-rabbit IgG (1:100: Jackson Immuno Labs, West Grove, PA) and fluorescein-conjugated affinity purified goat anti-mouse IgG (1:100: Jackson) for 1–2 h at room temperature. Sections were washed for 30 min with 0.1 M PB and coverslipped with 10% glycerol in 0.1 M PB containing 2% KI. To ensure that the secondary antibody had not cross-reacted with the inappropriate primary antibody, some sections were incubated in rabbit polyclonal primary antibody followed by anti-mouse secondary antibody, while other sections were incubated in mouse primary antibody followed by anti-rabbit secondary antibody. These sections did not show any immunostaining.

Topography and quantitation

The topography and quantitation of the NPY-immunoreactive cell populations were analyzed in two well-stained retinas. The data for the isodensity maps were plotted conventionally under a microscope. For the density maps, a field of 200×200 μm^2 was sampled in 1-mm steps over the full extent of the retina. Nearest-neighbor analysis (Wässle and Riemann 1978) was performed on the cells located in the mid-nasal region of the retina. Using an image analyzer (BMI-PLUS; Bummi Universe Co., Ansan, Korea), the soma size was measured in 60 cells from each population of NPY-IR cells, in both central and peripheral regions of the retina. The densities and soma sizes were averaged to give a representative mean value. The results were not corrected for shrinkage of the tissue during the mounting process, as this was negligible.

Electron microscopy

Three adult guinea pigs were anesthetized and put to death as described above. The eyecups were fixed in a mixture of 4% paraformaldehyde and 0.2% picric acid in PB for 30 min at room temperature. The retinas were then carefully dissected, and small pieces were taken from the central region and fixed for an additional 2 h at 4°C. After being washed in PB, the retinal pieces were transferred to 30% sucrose in PB for 6 h at 4°C, rapidly frozen in liquid nitrogen, thawed, and embedded in 4% agar in distilled water. The retinal pieces were sectioned with a vibratome to 40 µm, and the sections were placed in PBS. They were incubated in 10% NGS in PBS for 1 h at room temperature, to block nonspecific binding, and were then incubated in NPY antibody diluted 1:1000 for 12 h at 4°C.

The following immunocytochemical procedures were carried out at room temperature. The sections were washed in PBS for 45 min (3×15 min), incubated in biotin-labeled goat anti-rabbit IgG for 2 h, and then washed 3 times in PBS for 45 min (3×15 min). The sections were incubated in ABC solution for 1 h, washed in TB, and then incubated in 0.05% DAB solution containing 0.01% H_2O_2. The reaction was monitored using a low-power microscope, and was stopped by replacing the DAB and H_2O_2 solution with TB. The stained sections were postfixed in 1% glutaraldehyde in PB for 1 h. They were washed in PB containing 4.5% sucrose for 15 min, then postfixed in 1% OsO_4 in PB for 1 h, dehydrated in a graded series of alcohol, and flat-embedded in Epon 812. After the sections had been cured at 60°C for 3 days, well-stained areas were cut out and attached to an Epon support for further ultrathin sectioning using a Reichert-Jung ultratome. Ultrathin sections (70–90 nm thick) were collected on one-hole grids coated with Formvar, and examined by electron microscope (JEOL 1200EX, Tokyo, Japan).

Results

NPY immunoreactivity was observed in one morphologically distinct population of amacrine cells with somata located in the proximal row of the INL. No immunoreactivity was observed in the outer nuclear layer, the outer plexiform layer, or the GCL.

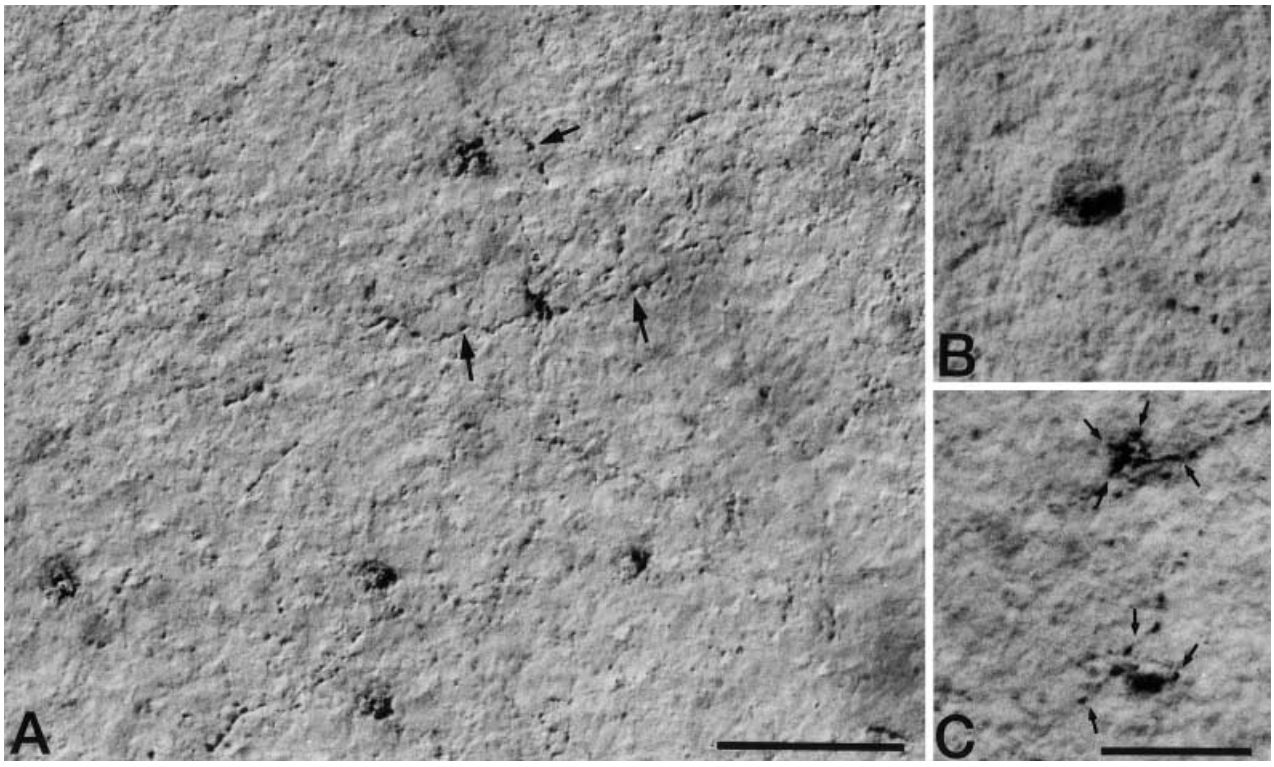

Fig. 1A–C Light micrographs taken at different focal planes of a whole-mount guinea pig retina, processed for neuropeptide Y (NPY) immunoreactivity. **A** The focus is at the inner plexiform layer (IPL) close to the inner nuclear layer (INL). Sparsely branched processes (*arrows*) with varicosities are seen. **B** The focus is at the INL. The NPY-labeled cell is round in shape. **C** The focus is at the IPL/INL border. Three or four primary processes (*arrows*) appear to emerge from the somata of the labeled amacrine cells. *Scale bar* 50 µm

In the whole-mount retinal preparations, when we focused on the INL, NPY-immunoreactive amacrine cell somata were distributed sparsely throughout the retina (Fig. 1). The labeled somata were round or oval. Three or four primary dendrites emerged from the labeled somata, and branched into several secondary dendrites with varicosities, ramifying horizontally in stratum 1 of the IPL. In a vertical vibratome section immunostained with anti-NPY antiserum, NPY immunoreactivity is mainly localized to the cytoplasm of a cell soma facing the IPL (Fig. 2A), and their processes ramify as puncta in stratum 1 of the IPL (Fig. 2B).

Soma sizes were measured from 60 NPY-labeled cells located in each of the central and peripheral regions of the whole-mount retina (Fig. 3). The mean diameter of

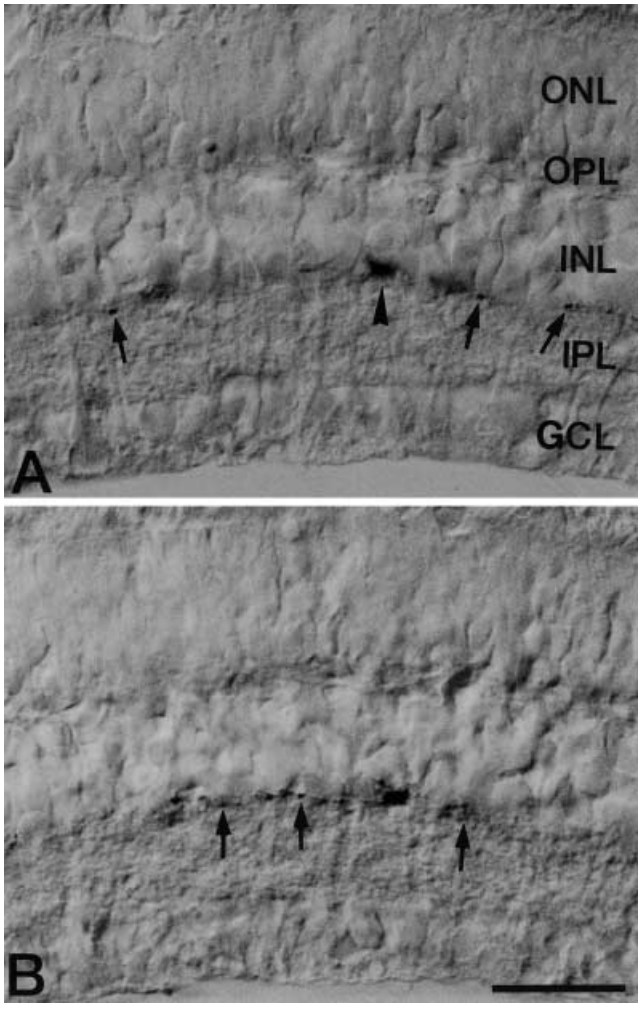

Fig. 2A, B Light micrographs taken at different focal planes of 50-µm-thick vertical vibratome sections processed for NPY immunoreactivity. **A** Strong NPY immunoreactivity (*arrowhead*) is localized to the cytoplasm of a cell facing the inner plexiform layer (*IPL*). *Arrows* indicate labeled processes. **B** The soma shown in **A** is out of focus. Labeled processes are visible in the IPL as puncta (*arrows*) (*ONL* outer nuclear layer, *OPL* outer plexiform layer, *INL* inner nuclear cell layer, *GCL* ganglion cell layer). *Scale bar* 50 µm

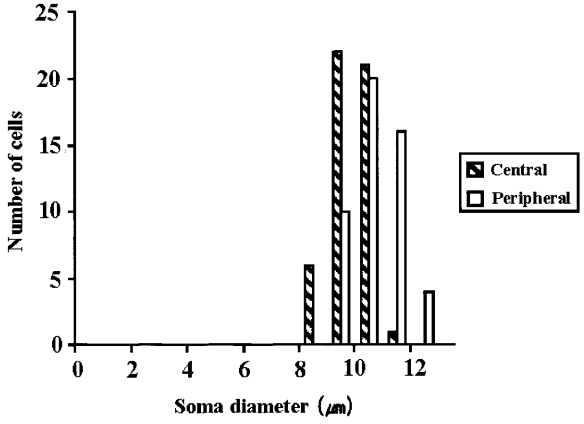

Fig. 3 Frequency/soma diameter graphs of NPY-labeled amacrine cells in the guinea pig retina. NPY-labeled amacrine cells were sampled from the INL at each of the central and peripheral regions of a whole-mount retina

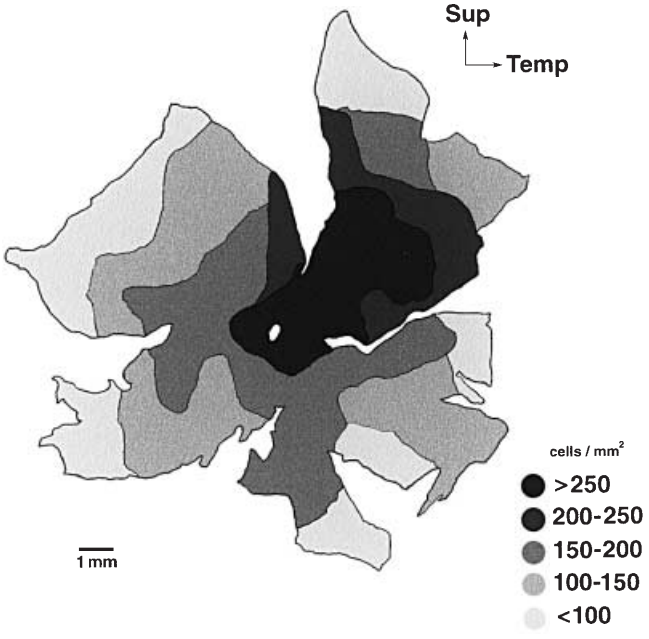

Fig. 4 Isodensity maps of the distribution of NPY-labeled amacrine cells in the INL of the same guinea pig retina (*Sup* superior, *Temp* temporal)

NPY-labeled cells was 9.8±0.7 μm in the central retina and 10.7±0.9 μm in the peripheral retina. The population properties of the NPY-labeled amacrine cells were evaluated in whole-mount preparations. The density peaked at 315±12 cells/mm^2 in the central region around the optic disk, and decreased toward the retinal periphery, with minimum densities of 98±23 cells/mm^2, and of about 160–250 cells/mm^2 elsewhere (Fig. 4).

As shown in Fig. 1A, the somata of labeled amacrine cells are rather irregularly distributed. To assess the distributions quantitatively, we used a nearest-neighbor analysis (Wässle and Riemann 1978). The distance of

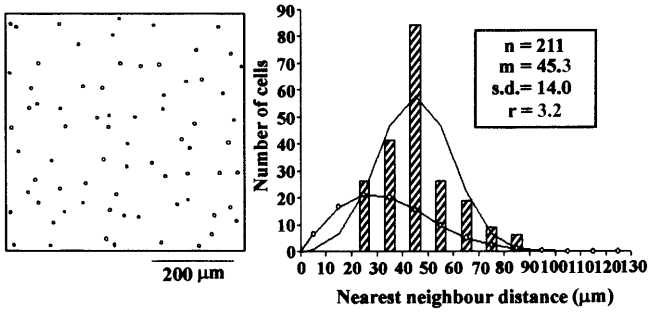

Fig. 5 Nearest-neighbor analysis of the NPY-immunoreactive amacrine cell populations in the INL. *Left* Drawing of part of the analyzed field in the mid-peripheral nasal region of the retina. The labeled cell population appears relatively regularly spaced. *Right* Nearest-neighbor distance histogram for the labeled amacrine cells (*hatched*). The histogram for the labeled cell population is relatively matched by the Gaussian curve (*solid line*), which describes a regular cell distribution, but not by the Poisson curve (*dashed line with circles*), which describes a random cell distribution (Rayleigh distribution). The histogram shows the number of cells in the sample (*n*), mean distance between cells (*m*), standard deviation (*s.d.*), and regularity index (*r*)

each soma to its nearest neighbor was measured in the mid-peripheral field of the retina: 1×1 mm^2 in size. The resulting histograms for the NPY-labeled cells are shown (Fig. 5, right). The histogram for the labeled amacrine cells is fairly well fitted by a Gaussian distribution with the same mean distance and standard deviation (Fig. 5, solid lines), indicating a statistically regular mosaic.

To identify whether NPY- and GABA are co-localized within the same amacrine cells, double-labeling experiments were performed (Fig. 6). Immunostaining using GAD_{65} antibodies was similar to earlier reports (Agardh et al. 1987; Vardi and Auerbach 1995), with numerous GAD_{65}-immunoreactive amacrine cells characterized by prominent staining of the cytoplasm and dense processes in the IPL. All NPY-labeled amacrine cells (*n*=65) investigated in the guinea pig retina also showed GAD_{65} immunoreactivity. Therefore, we conclude that all NPY-immunoreactive amacrine cells constitute a subpopulation of GABA-ergic amacrine cells.

NPY immunoreactivity was easily identified by the presence of electron-dense reaction products. The reaction product was closely associated with mitochondrial membranes, the nuclear membrane, amorphous matrices of the cytoplasm, and synaptic vesicles.

From previous descriptions of processes and synapses in the IPL, the following criteria were applied (Dubin 1970; Kolb 1979; McGuire et al. 1984, 1986). Terminals that contained synaptic ribbons were considered to belong to bipolar cells. Processes that contained synaptic vesicles and made conventional chemical output or electrical synapses were considered to be amacrine cell processes, and processes that contained microtubules instead of synaptic vesicles were defined as ganglion cell dendrites.

Synapses made by the NPY-labeled processes were exclusively observed in stratum 1 of the IPL (summa-

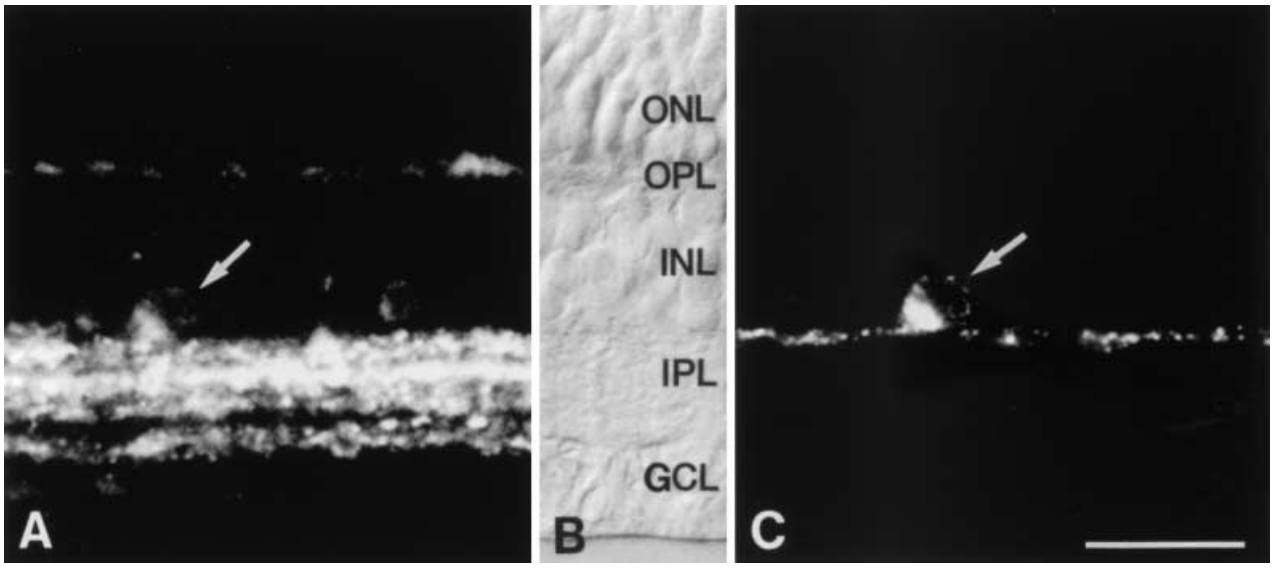

Fig. 6 Confocal micrographs taken from a vertical vibratome section processed for GAD_{65} **(A)** and NPY **(C)** immunoreactivities. GAD_{65} immunoreactivity was visualized using a fluorescein-conjugated secondary antibody; NPY immunoreactivity was visualized using a Cy3-conjugated secondary antibody. **A** Two GAD_{65}-immunoreactive cell bodies are seen at the inner margin of the inner nuclear layer (*INL*). The inner plexiform layer (*IPL*) contains a dense network of labeled processes. **B** A vertical vibratome section showing well-organized layers. **C** A NPY-immunoreactive cell body is visible in the INL. *Arrowheads* indicate labeled processes in the IPL. A cell body displaying both immunoreactivities is indicated by *arrows*. *Scale bar* 25 µm

Table 1 Kinds of synapses made by NPY-immunoreactive neurons in the inner plexiform layer of the guinea pig retina

	Sublamina a		Sublamina b			Total (%)
	S1	S2	S3	S4	S5	
Input from:						
Amacrine cells	65	0	0	0		65 (81.2)
Bipolar cells	15	0	0	0	0	15 (18.8)
Subtotal	80	0	0	0	0	80 (100)
Output onto:						
Ganglion cells	0	0	0	0	0	0
Amacrine cells	54	0	0	0	0	54 (69.2)
Bipolar cells	11	0	0	0	0	11 (14.1)
Unidentified	13	0	0	0	0	13 (16.7)
Subtotal	78	0	0	0	0	78 (100)
Labeled to labeled	0	0	0	0	0	0
Total	158	0	0	0	0	158

rized in Table 1). Labeled processes were filled with synaptic vesicles, closely related to the electron-dense reaction products. Because of numerous vesicles and the lack of synaptic ribbons, NPY-labeled processes could be easily identified as amacrine cell processes.

A total of 158 synapses were observed in stratum 1 of the IPL. No synapses were visible in strata 2, 3, 4 and 5 of the IPL. Of these, 80 were synapses with NPY-immunoreactive amacrine cell processes made by unlabeled amacrine cell processes and bipolar axon terminals. A total of 78 synapses were NPY-labeled amacrine cell processes synapsing onto amacrine cells, bipolar cells, and unidentified cells.

The most common synaptic input to NPY-immunoreactive amacrine cells is from unlabeled amacrine cell processes (81% of all synaptic input synapses in stratum 1: n=80). Figure 7A shows a labeled amacrine cell process receiving conventional synaptic input from an unlabeled amacrine cell process. Synaptic input from bipolar cell axon terminals to NPY-immunoreactive amacrine cell processes was less commonly observed. It comprised 19% (n=15) of all synaptic input. In most cases of the bipolar axon terminals, two postsynaptic elements at ribbon synapses were observed (Fig. 7B). One of these was the NPY-immunoreactive amacrine cell process and the other was either an unlabeled amacrine cell process or a ganglion cell dendrite.

NPY-immunoreactive amacrine cell processes formed conventional output synapses to amacrine cells, bipolar cells and unidentified processes in 78 of the 158 synapses observed in our study. Of the output synapses of NPY-labeled amacrine cells, 69% (n=54) were to amacrine cells. Figure 8A, B shows a labeled amacrine cell process that is presynaptic to an unlabeled amacrine cell process. Figure 8C shows an example of a synapse to a small process (U) that is classified as "unidentified" (the process is too small to be identified as either a ganglion cell dendrite or an amacrine cell process). The incidence of synapses with unidentified processes was 17% (n=13) of all output synapses. Synaptic output from NPY-labeled amacrine cell processes to bipolar cell axons, or axon terminals, comprise 14% (n=11) of all output synapses found. Type 1 OFF cone bipolar cells, identifiable by the nature of their postsynaptic dyads and their location in the IPL (Euler and Wässle 1995), were postsynaptic to the labeled processes. In Figure 8D, a bipolar axon terminal with a synaptic ribbon is postsynaptic to a NPY-immunoreactive amacrine cell process.

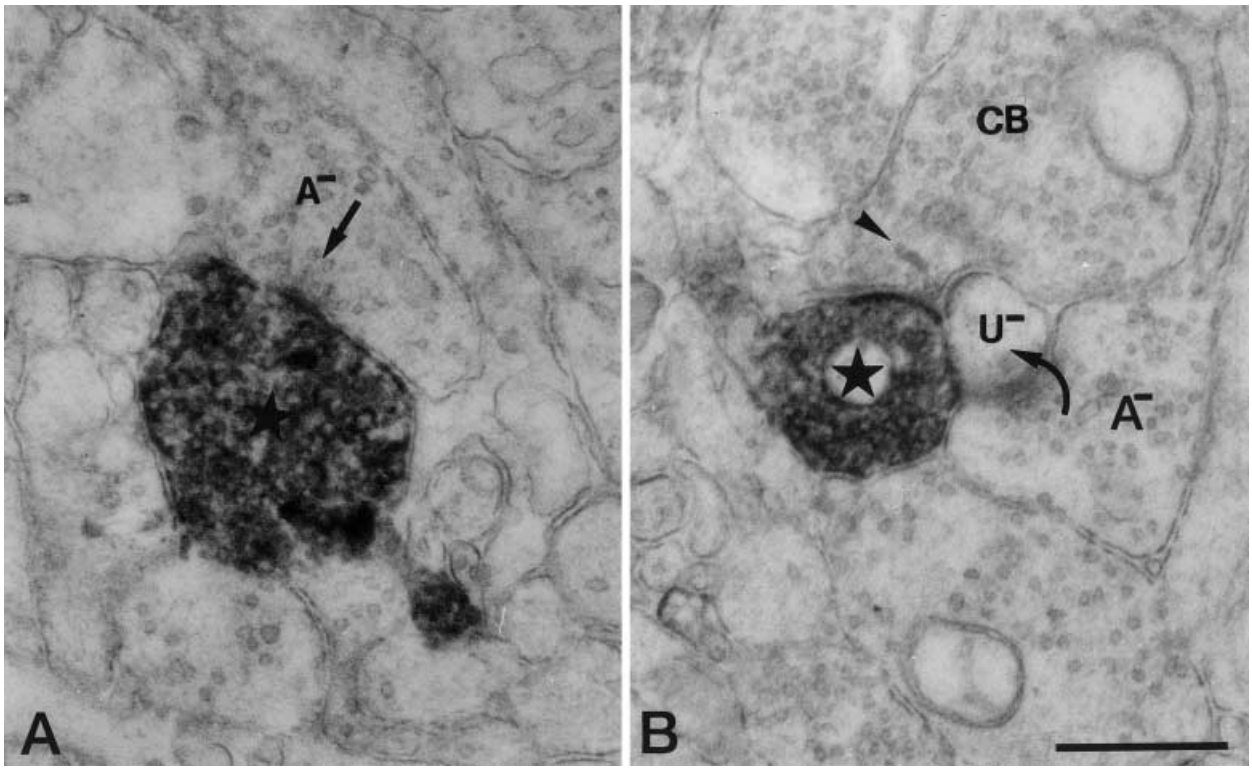

Fig. 7A, B Vertical ultrathin sections through the stratum 1 of the IPL of the guinea pig retina processed for NPY immunoreactivity. **A** A labeled amacrine cell process (*star*) receives synaptic input (*arrow*) from an unlabeled amacrine cell process (*A*-). **B** A labeled amacrine cell process (*star*) forms a postsynaptic dyad with an unlabeled cell process (*U*-), which is postsynaptic (*curved arrow*) to an unlabeled amacrine cell process (*A*-) at the ribbon synapse (*arrowhead*) of a cone bipolar axon terminal (*CB*) in stratum 1 of the IPL. *Scale bar* 0.5 µm

Discussion

The present study characterizes the morphology, distribution, and synaptic connectivity of NPY-immunoreactive neurons of the guinea pig retina. Based on their cellular appearance and distribution, NPY-immunoreactive neurons are amacrine cells with widely ramifying processes in stratum 1 of the IPL, although their processes are shown as a series of puncta. These cells are widely and relatively regularly spaced in the INL.

The distribution of NPY-immunoreactive neurons has been studied in the retina of several mammals, including man (Tornqvist and Ehinger 1988; Li and Lam 1990; Jen et al. 1994; Jotwani et al. 1994), rat (Ferriero and Sagar 1989; Oh et al. 1999), mouse (Sinclair and Nirenberg 2001), guinea pig (Bruun et al. 1984), baboon (Bruun et al. 1986), cat (Hutsler et al. 1993; Hutsler and Chalupa 1994, 1995), and monkey (Marshak et al. 1986). Our findings are in agreement with the findings from a previous study of the guinea pig retina, which reported that NPY-immunoreactive fibers form a single layer of processes in stratum 1 of the IPL and NPY-immunoreactive cell bodies are located in the innermost cell row of the INL (Bruun et al. 1984). In addition, these immunoreactive cells are remarkably similar to NPY-labeled cells described in the rat (Oh et al. 1999), mouse (Sinclair and Nirenberg 2001), cat (Hustler and Chalupa 1994) and human (Tornqvist and Ehinger 1988; Straznicky and Hiscock 1989; Jotwani et al. 1994), in that the main population of NPY-labeled cells is located in the innermost row of the INL and ramifies in stratum 1 of the IPL, the presumptive OFF sublamina (Famiglietti and Kolb 1976; Nelson et al. 1978; Peichl and Wässle 1981). However, in other mammalian species, other types of NPY-labeled cells have been reported in addition to the main NPY-labeled amacrine cells ramifying in stratum 1 of the IPL. For instance, in the rat retina (Oh et al. 1999), two other types of NPY-labeled amacrine cells have been identified: amacrine cells with ramifying in stratum 3 of the IPL and displaced amacrine cells with processes ramifying in stratum 5 of the IPL. In the mouse retina, displaced amacrine cells with processes ramifying in the IPL close to the GCL are present (Sinclair and Nirenberg 2001). In the monkey retina, amacrine cells with processes ramifying in strata 1 and 5 have been reported to show NPY immunoreactivity (Marshak et al. 1986). In addition, NPY-immunoreactive ganglion cells have also been reported in the cat and human retina (Straznicky and Hiscock 1989; Hutsler and Chalupa 1994). This diversity of morphological types of NPY-immunoreactive cells in mammalian retinas suggests that these cells have different roles in the retinas of different species.

NPY-immunoreactive amacrine cells in the guinea pig retina were characterized by double-label immunohistochemistry with a marker for GABA-ergic amacrine cell

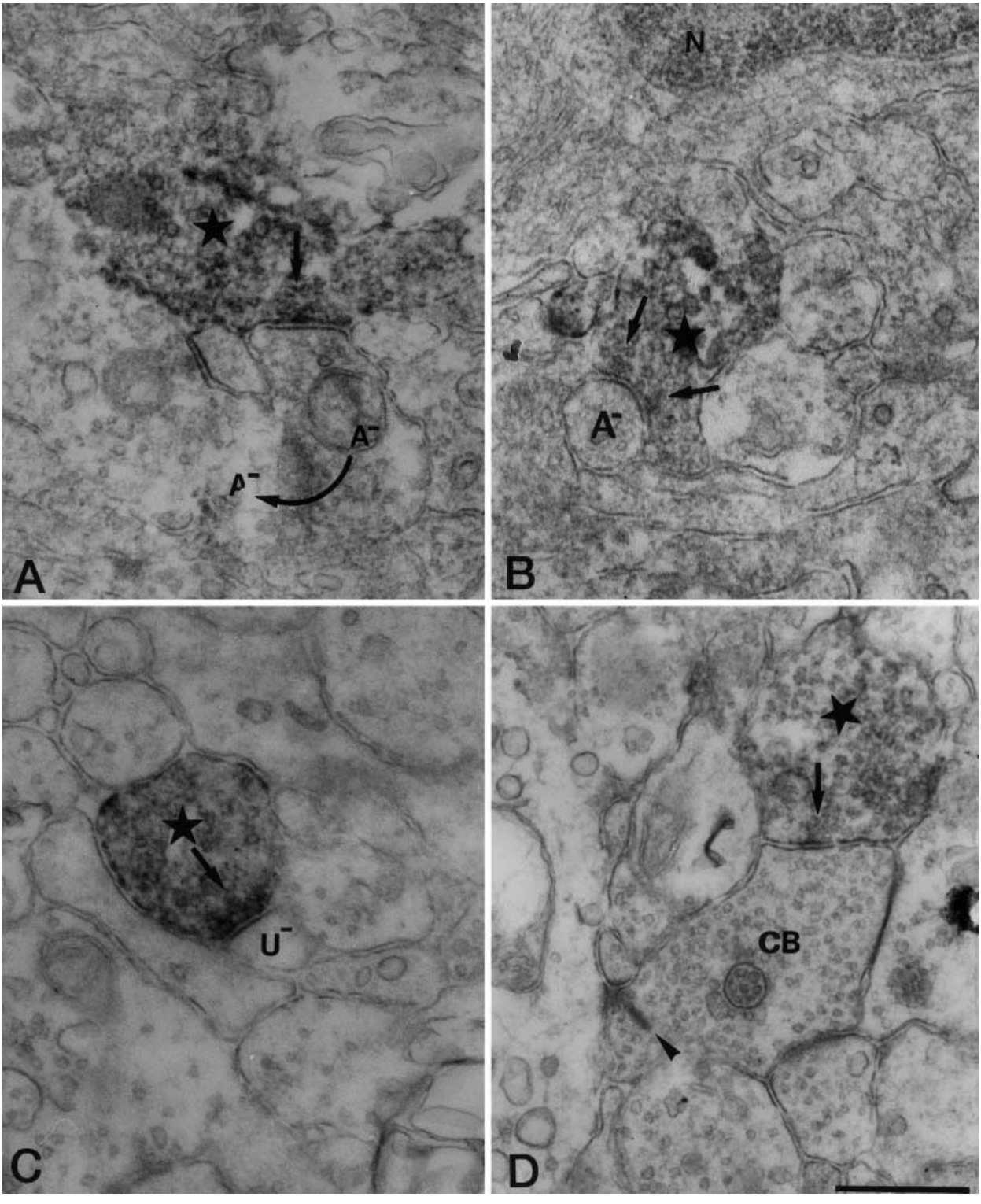

Fig. 8A–D Vertical ultrathin sections through the stratum 1 of the IPL of the guinea pig retina processed for NPY immunoreactivity. **A** A labeled amacrine cell process (*star*) makes a chemical output synapse (*arrow*) with an unlabeled amacrine cell process (*A*-), which in turn makes a synapse (*curved arrow*) onto an unlabeled amacrine cell process (*A*-). **B** A labeled amacrine cell process (*star*) making an output synapse (*arrows*) onto an unlabeled amacrine cell process (*A*-) is seen. **C** A labeled amacrine cell process (*star*) makes synaptic output (*arrow*) onto an unidentified cell process (*U*-). **D** A labeled amacrine cell process (*star*) makes synaptic output (*arrow*) onto a cone bipolar axon terminal (*CB*) with a synaptic ribbon (*arrowhead*). *Scale bar* 0.5 µm

populations in the present study. All NPY-immunoreactive amacrine cells contained GAD65 immunoreactivity, demonstrating that the NPY-labeled amacrine cells comprise a subpopulation of GABA-containing amacrine cells. These results are in good agreement with previous studies, in which NPY-immunoreactive amacrine cells show GAD_{65} immunoreactivity in the rat retina (Oh et al. 1999) or GABA transporter-1 immunoreactivity in the mouse retina (Sinclair and Nirenberg 2001). The co-localization of bioactive peptides, including VIP and substance P, with GABA has been reported in wide-field amacrine cells in other mammalian retinas (Vaney et al. 1989; Casini and Brecha 1992). The present findings are therefore consistent with previous findings and further support the idea that a general organizational feature of the mammalian retina is the coexpression of a biologically active substance with GABA in wide-field amacrine cells. The co-localization of NPY and GAD_{65} immunoreactivities in the same cells indicates that NPY-containing amacrine cells may also use GABA as a transmitter. The functional significance of this co-localization is still unknown. In other systems, NPY and GABA may be released by different stimulus intensity or patterns, and they may act independently or in concert, on target sites (Hökfelt 1991; Parker et al. 1998; Pu et al. 1999). We have shown that NPY-immunoreactive amacrine cells form conventional chemical synapses, suggesting that GABA is indeed released as a regular transmitter at these sites and may have typical inhibitory effects on the postsynaptic cells. Thus, co-released NPY might modulate the action of its co-transmitter in the retina. However, it cannot be excluded that NPY might diffuse within the extracellular space and influence neighboring neurons that are not directly postsynaptic to NPY-labeled amacrine cells, in a paracrine manner (D'Angelo and Brecha 1999). This paracrine mode of action for NPY is further supported by observations on the differential distribution of NPY-containing processes and their receptors in the retina (Ammar et al. 1998). Thus, further investigations are clearly needed to elucidate the precise role of NPY in synaptic transmission in the retina.

In the present study, NPY-immunoreactive amacrine cell processes receive their major synaptic input from unlabeled amacrine cell processes and bipolar cell axonal terminals in stratum 1 of the IPL. Based on these findings, NPY-immunoreactive amacrine cells are modulated by other amacrine cells and type 1 cone bipolar cells (Euler and Wässle 1995).

NPY-immunoreactive amacrine cell processes make conventional synaptic contacts with both unlabeled amacrine cells and bipolar cells in strata 1 of the IPL. These results suggest that the predominant site of action for NPY released from NPY-immunoreactive cells is on amacrine cells. The synaptic connectivity of NPY-labeled amacrine cells is quite similar to that of nitric oxide synthase-immunoreactive amacrine cells, of which amacrine cells are frequent output targets (Chun et al. 1999; Oh et al. 1999). Together with these reports, it can be inferred that the NPY-immunoreactive amacrine cells might be inter-amacrine cells that may be modulated by, and affect other amacrine cells. The kind of bioactive substances that the amacrine cells, postsynaptic to NPY-immunoreactive processes, use as their transmitters is unknown. However, there are some clues as to the organization of these inner retinal pathways: an earlier pharmacological study, in the rabbit retina, showed that endogenously applied NPY results in the release of dopamine, glycine and serotonin from the retina (Bruun and Ehinger 1993), indicating an action of NPY on several amacrine cell types, including the dopamine- and serotonin-containing wide-field amacrine cells (Brecha et al. 1984; Hokoc and Mariani 1987; Mitrofanis et al. 1988; Dacey 1990; Tauchi et al. 1990) and one or more of the glycine-containing narrow field amacrine cell types (Pourcho and Goebel 1985; Hendrickson et al. 1988; Wright et al. 1997; Menger et al. 1998). However, further experiments are clearly needed to elucidate the identity of pre- or postsynaptic amacrine cells to NPY-immunoreactive cells in the mammalian retina.

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