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# NPY in rat retina is present in neurons, in endothelial cells and also in microglial and Müller cells

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**Abstract**

NPY is present in the retina of different species but its role is not elucidated yet. In this work, using different rat retina in vitro models (whole retina, retinal cells in culture, microglial cell cultures, rat Müller cell line and retina endothelial cell line), we demonstrated that NPY staining is present in the retina in different cell types: neurons, macroglial, microglial and endothelial cells. Retinal cells in culture express NPY $Y_1$, $Y_2$, $Y_4$ and $Y_5$ receptors. Retina endothelial cells express all NPY receptors except NPY $Y_5$ receptor. Moreover, NPY is released from retinal cells in culture upon depolarization. In this study we showed for the first time that NPY is present in rat retina microglial cells and also in rat Müller cells. These in vitro models may open new perspectives to study the physiology and the potential pathophysiological role of NPY in the retina.


*Keywords:* Neuropeptide Y (NPY); Rat retina; Microglial cells; Müller cells; Endothelial cells; NPY receptors; NPY release

## 1. Introduction

The retina is a very organized structure of the nervous tissue that contains two main classes of cells: neurons and glial cells. Retinal neurons include photoreceptors, bipolar, ganglion, horizontal and amacrine cells that are strategically localized in various layers through. Glial cells comprise macroglial and microglial cells. Two main macroglial elements are present in the retina (Newman, 2004): Müller cells that span the entire thickness of the retina, give the support to the retina by regulating retinal metabolism and modulate the function of neurons and blood vessels; astrocytes are associated with optic nerve axons and retinal blood vessels. Microglia is normally quiescent but very sensitive to the retina homeostatic state. When the homeostasis is disturbed, microglia rapidly acts as phagocytes, or release inflammatory mediators (Chen et al., 2002).

Neuropeptide Y (NPY) is a highly conserved, 36 amino acid peptide, widely distributed in the central nervous system (Silva et al., 2005b) being also present in the retina of different species (Bruun et al., 1986; Hutsler and Chalupa, 1995; Jen et al., 1994; Oh et al., 2002). NPY-ir is mainly localized in amacrine cells but it is also present in ganglion cells (Ammar et al., 1998; Hutsler and Chalupa, 1994; Sinclair and Nirenberg, 2001). In rat retina, NPY-ir is present in two populations of amacrine cells, one located in the inner nuclear layer and the other in the ganglion cell layer. Little is known about the function of NPY system in the retina but some observations propose that NPY-ir cells participate in multiple circuits mediating visual information processing in the inner retina. It was suggested that these cells are involved in spatial tuning, specifically, in tuning ganglion cells to low spatial frequencies (Sinclair et al., 2004). In addition, exogenous application of NPY stimulates the release of glycine, dopamine, and 5-hydroxytryptamine from the rabbit retina, and gamma-aminobutyric acid (GABA) and

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choline from the chicken retina, suggesting that NPY modulates neurotransmitter release in the retina (Bruun and Ehinger, 1993). All these observations suggest that NPY is probably released in response to visual stimulus. However, the conditions required for NPY release must be investigated. The fact that NPY is released by high frequency light stimulation or depolarization was only demonstrated only in the frog retina (Bruun et al., 1991). The release of NPY in the retina of other vertebrates was not yet demonstrated.

NPY mediates its effects through the activation of six G-protein-coupled receptor subtypes named $Y_1$, $Y_2$, $Y_3$, $Y_4$, $Y_5$, and $y_6$. Five distinct NPY receptors have been cloned, $Y_1$, $Y_2$, $Y_4$, $Y_5$ and $y_6$ (Silva et al., 2002). The $Y_3$ receptor has not yet been cloned while $y_6$ receptor is not functional (Michel et al., 1998). The presence of mRNA for the $Y_1$, $Y_2$, $Y_4$ and $Y_5$ receptors was detected in intact rat retinas (D'Angelo and Brecha, 2004).

Endothelial cells are another cell type that forms an important structure of the retina: blood-retinal-barrier (BRB). This structure separates the retina from the blood circulation by a barrier located both at the retinal vessels, the inner BRB, and at the retinal pigment epithelium, the outer BRB. NPY has been reported to directly stimulate endothelial cell proliferation and migration (Ekstrand et al., 2003; Ghersi et al., 2001; Marion-Audibert et al., 2000; Zukowska-Grojec et al., 1998a,b). In the retina, some evidences have shown that NPY and $Y_2$ receptors are involved in development of diabetic retinopathy and retinal neovascularization (Koulu et al., 2004).

Several lines of evidence suggest that the expression of NPY and NPY receptors, in central nervous system, may not be restricted to neurons, but could also extend to glial cells, namely in astrocytes (Barnea et al., 1998; Gimpl et al., 1993; St-Pierre et al., 2000). The presence of NPY in retina glial cells, such as in microglial cells and in Müller cells, was not yet investigated.

The aim of the present study was to better characterize the presence of NPY on different in vitro rat retina models. Therefore, whole rat retinas, primary cell cultures of rat retinal cells, primary cell cultures of retina microglial cells, a cell line of rat Müller cells, and a cell line of rat retina endothelial cells were used. The mRNA for NPY and NPY receptors were detected in whole rat retinas, primary cell cultures of rat retinal cells and in a cell line of rat retina endothelial cells. Moreover, the NPY release was evaluated using the primary culture of rat retinal cells. The development and characterization of new in vitro models of the retina will allow defining the role of NPY in retinal physiology and pathophysiology.

## 2. Materials and methods

### 2.1. Materials

Minimum essential medium (MEM) was purchased from Sigma Chemical Co., St. Louis, MO, USA. Dulbecco's modified Eagle medium (DMEM) and trypsin were purchased from GIBCO BRL, Life Technologies, Scotland, UK. Taq DNA polymerase was purchased from Amersham Biosciences Europe GmbH, Freiburg, Germany. First strand cDNA Synthesis Kit for RT-PCR (AMV) was purchased from Roche Applied Science, PO, Indianapolis, USA. Fetal bovine serum was obtained from Biochrom, Berlin, Germany.

### 2.2. Cell cultures

#### 2.2.1. Primary rat retina neural cell culture

Three to 5 days old Wistar rats were used to prepare primary rat retina cell culture as previously described (Santiago et al., 2006). All procedures involving animals were in agreement with the Association for Research in Vision and Ophthalmology (ARVO) statement in vision and ophthalmic research. Briefly, rat retinas were digested with 0.1% trypsin (w/v) for 15 min at 37 °C. Cells were plated on poly-D-lysine (0.1 mg/ml) coated wells with MEM, supplemented with 25 mM HEPES, 26 mM $NaHCO_3$, 10% FCS and penicillin (100 U/ml)–streptomycin (100 μg/ml) for 9 days (37 °C, 5% $CO_2$), at a density of $2 \times 10^6$ cells/cm$^2$. For immunocytochemistry experiments, cells were plated on glass coverslips and for RNA extraction cells were plated on 35 mm Petri dishes at the same density. For NPY assays retina neural cell cultures were plated on 60 mm Petri dishes at density of $3 \times 10^6$ cells/cm$^2$.

#### 2.2.2. Rat retina microglial cell culture

Primary rat microglial cell cultures were prepared from 3 to 5 days old Wistar rats retinas as described with some modifications (Wang et al., 2005). Briefly, retinas were dissected under sterile conditions using a light microscope in $Ca^{2+}$- and $Mg^{2+}$-free Hank's balanced salt solution (HBSS, in mM: 137 NaCl, 5.4 KCl, 0.45 $KH_2PO_4$, 0.34 $Na_2HPO_4$, 4 $NaHCO_3$, 5 glucose; pH 7.4). The retinas were digested in HBSS supplemented with 0.1% trypsin (w/v) and 0.001% DNase (w/v), for 15 min at 37 °C. After dissociation, trypsin was inactivated by 10% heat-inactivated fetal bovine serum (FBS) and the cells were pelleted by centrifugation. The cells were resuspended in DMEM/F-12 HAM supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, and plated at a density of $1.5 \times 10^6$ cells/cm$^2$ on 75 cm$^2$ growth surface area plastic flasks coated with poly-D-lysine (0.1 mg/ml). The cells were kept in culture at 37 °C under a 95% air/5% $CO_2$ atmosphere and the medium was changed every 3 days. After 2 weeks, microglia cells were harvested in culture medium containing serum by shaking the flasks at 150 rpm for 30 min. The detached cells were plated at a density of $25 \times 10^4$ cells/cm$^2$ on 16 mm glass coverslips coated with poly-D-lysine (0.1 mg/ml). The cells were used for immunocytochemistry experiments after 3 days in culture.

#### 2.2.3. Culture of rat retina endothelial cell line—TR-iBRB

Conditionally immortalized retinal capillary endothelial cell line (TR-iBRB—transgenic rat-inner blood-retinal barrier) was obtained from Dr. Ken-ichi Hosoya, and cultured as previously described (Hosoya et al., 2001). These cells were plated on glass coverslips and cultured in DMEM at 33 °C/5% $CO_2$ for 3–4 days until 80–90% of confluence.

#### 2.2.4. Culture of rat retina Müller cell line

Conditionally immortalized rat Müller cell line (TR-MUL—transgenic rat Müller cells) were cultured as previously described (Tomi et al., 2003). Müller cells were plated on glass coverslips and cultured in DMEM at 33 °C/5% $CO_2$ for 3–4 days until 80–90% of confluence.

### 2.3. Determination of NPY content and release in rat retina cell cultures

Retina cells in culture were washed three times with Krebs buffer (132 mM NaCl, 4 mM KCl, 1.4 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES, pH 7.4) containing Tween 20 (0.0001%) in order to prevent NPY sticking to plastic. After 20 min of pre-incubation, at room temperature in Krebs buffer, the cells were incubated with Krebs buffer for 10 min at room temperature with or without 50 mM KCl. NPY released without KCl (constitutive release) and with KCl was measured by an ELISA assay (described below). NPY intracellular content in whole retina or in cell cultures were determined in Krebs buffer with 50 mM EDTA and 0.0001% Tween 20 and after freeze–defreeze, and sonication for 12 s. The cell suspension was centrifuged (14,000 rpm/5 min/4 °C). NPY (released and content) was measured using a NPY sandwich-ELISA assay as previously described (Grouzmann et al., 1992a). Each well from 96 wells microplate (PolySorp, Nunc) was coated with 200 ng (100 μl) of the monoclonal antibody NPY02 diluted in Tris buffer (50 mM, pH 7.5) for 16 h at 4 °C.

The plates were washed three times with Tris buffer containing 0.08% Tween 20 (Sigma) (Tris–Tween buffer) and incubated for 30 min with 200 μl of Tris–Tween 0.08% buffer containing 50 g/l low-fat dry milk. After washing four times with Tris–Tween 0.08% buffer, wells were filled with standards (standards ranging from 0.5 to 100 pM) and samples. The samples and standards were incubated overnight at RT. After washing four times with Tris–Tween 0.08% buffer a second antibody, NPY05 conjugated with alkaline phosphatase, diluted (1:4000) in 5% low-fat milk in Tris–Tween 0.08% buffer, was added (7 h at room temperature). The plates were then washed twice with Tris buffer containing 0.25% Tween 20 followed by washing two times with Tris buffer with 150 mM NaCl. Bound alkaline phosphatase was revealed by the addition of 50 μl of the substrate (NADPH solution from ELISA amplification kit, Invitrogen Life Technologies, Carlsbad, CA, USA). The amplifier (50 μl) (ethanol dehydrogenase mixed with diaforase from ELISA amplification kit, Invitrogen Life Technologies, Carlsbad, CA, USA) was added 45 min later, according to the manufacturer recommendations. The optic density was read at 492 nm (ELISA reader, SLT, Spectra II) for 30 min. The detection limit was 0.5 pM. This NPY assay measures biologically active forms of NPY ($NPY_{1-36}$, $NPY_{2-36}$ and $NPY_{3-36}$) (Grouzmann et al., 1992a). The protein content was measured by Bradford assay (Biorad) (Bradford, 1976).

### 2.4. Immunocytochemistry

Cells on glass coverslips were washed twice with PBS and fixed in 4% paraformaldehyde (30 min; room temperature). Then, the cells were incubated for 5 min with 1% Triton, and for 1 h with 3% (w/v) fatty acid-free bovine serum albumin (BSA), supplemented with 0.2% Tween 20, to prevent non-specific binding, at room temperature. Cells were incubated with primary antibodies for 90 min at room temperature: rabbit anti-Microtubule Associated Protein 2 (MAP-2; 1:500; Chemicon International, Temecula, CA, USA); mouse anti-NPY (NPY02; 1:1500; Dr. Eric Grouzmann, CHUV, Lausanne, Switzerland; Grouzmann et al., 1992b); rabbit anti-glial-fibrilliary acid protein (GFAP; 1:500; Dako, Denmark) or rat anti-macrophage antigen-1 (Mac-1, 1:500; Serotec, Oxfordshire, UK). After washing, the cells were incubated for 1 h at room temperature with secondary antibodies (1:200 Alexa™ 488 anti-mouse IgG, 1:150 Alexa™ 594 anti-rat IgG or 1:200 Alexa™ 594 anti-rabbit IgG; Invitrogen, Eugene, Oregon, USA). Cells were mounted using a Prolong Antifade Kit and visualized using a fluorescent microscopic (Zeiss Axioshop 2 Plus) coupled to a digital camera (Axiocam HRc).

### 2.5. RNA extraction and reverse transcriptase-PCR

Total RNA was isolated from the intact retina, neural and endothelial cell cultures using TRI Reagent (Sigma). The total amount of RNA was quantified by optical density (OD) measurements at 260 nm, and the purity was evaluated by measuring the ratio of OD at 260 and 280 nm (RNA/DNA calculator GeneQuantII, Pharmacia Biotech Amersham Biosciences AB, Uppsala, Sweden). First strand cDNAs were synthesized by incubating 2 μg of the total RNA from the neural and endothelial cell culture, or from intact retina with random

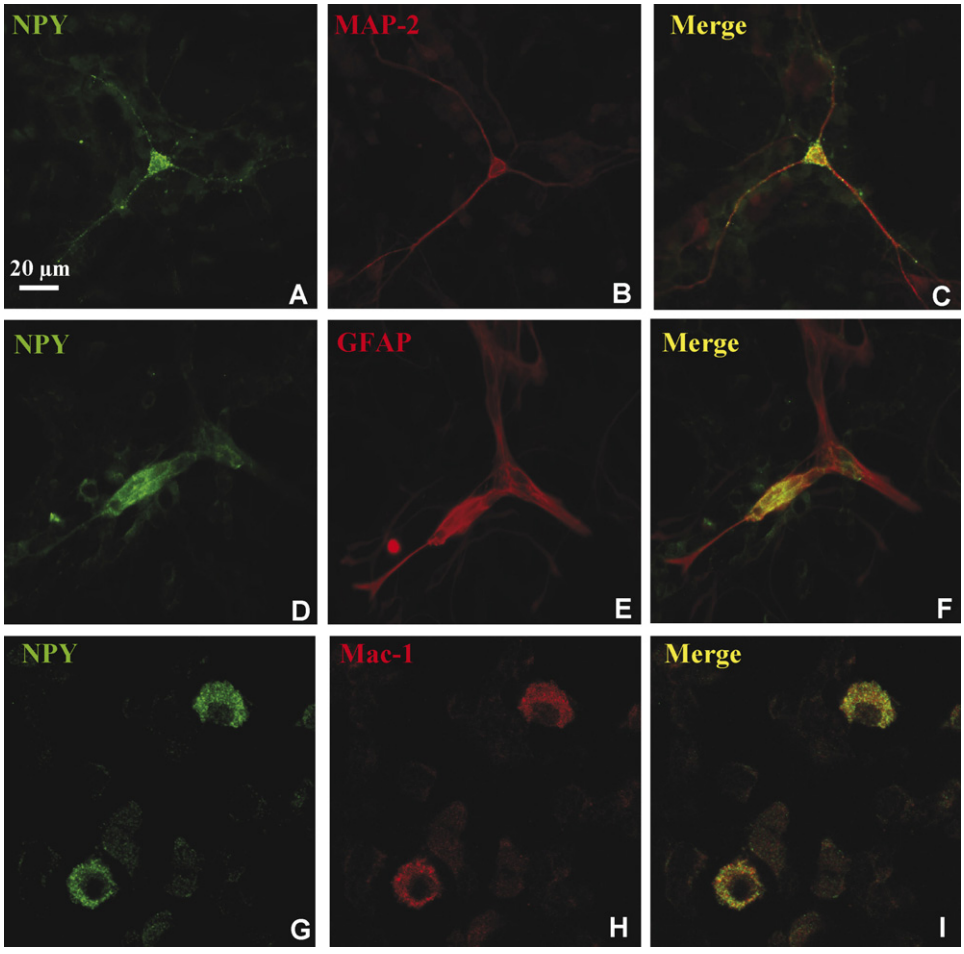


Fig. 1. NPY staining in different types of rat retinal cells in culture (A–I): double staining with neuronal, macroglial and microglial cell markers. NPY was detected (green) with a monoclonal mouse anti-NPY antibody (A, D, G). Retinal cells (A–I) were double stained with NPY and neuronal, macroglial or microglial cell markers. Neurons were labelled (red) with an anti-MAP-2 antibody (B and C); astrocytes and Müller cells were identified (red) with an anti-GFAP antibody (E and F); microglial cells were labelled (red) with an anti-Mac-1 antibody (H and I). The images are representative of three independent cell cultures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

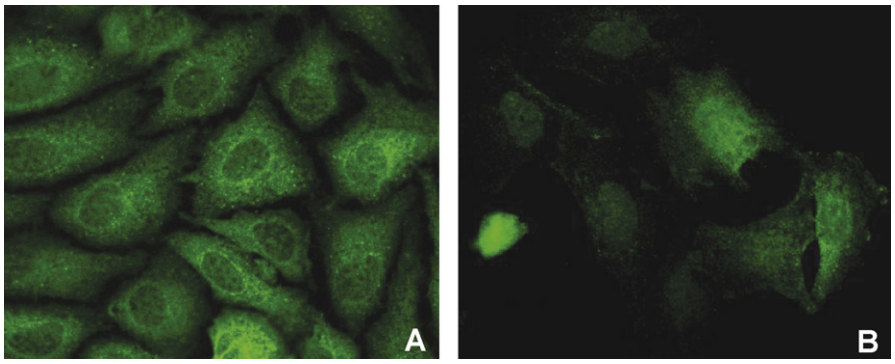


Fig. 2. NPY staining in rat retina endothelial cells (A) and in rat Müller cells (B). Rat retina endothelial cells (TR-iBRB cell line) and rat Müller cells (TR-mull cell line) were labelled with anti-NPY antibody. The images are representative of three independent cell cultures. 600×.

oligo (dT) primers, RNase inhibitor, and Avian Microblastosis Virus (AMV) reverse transcriptase (RT) for 1 h at 42 °C.

Reverse transcriptase-PCR (RT-PCR) was performed using specific primer pairs for rat NPY and NPY receptors. The primers were (forward and reverse and product size: NPY, 5′-AGAGATCCAGCCCTGAGACA-3′, 5′-AACGACAACAAGGGAAATGG-3′ (236 bp); $Y_1$, 5′-AACCTCTCCTTCTCAGACTTGC-3′, 5′-CACAGTGTTGAAGATGGTAAGG-3′ (616 bp); $Y_2$, 5′-CTCCAAGCAAATCAGCTTCC-3′, 5′-GTTTTGTGCCTTCGCTGATGG-3′ (318 bp); $Y_4$, 5′-AACCTACTCATTGCCAACCTG-3′, 5′-ATGTAGCAGACCAGGATGAAG-3′ (476 bp); $Y_5$, 5′-GTGTTCCCGAGGTGCTTCTA-3′, 5′-ATTCCGAGCAGCAGCTGTAT-3′ (248 bp) (MWG-Biotech AG, Ebersberg, Germany). Positive controls were plasmids encoding $Y_1$, $Y_2$, and $Y_5$ and rat brain cDNA (for NPY and $Y_4$). Negative controls were performed without RNA sample, which was substituted by water. All amplifications were performed in PCR buffer containing 1.5 mM $MgCl_2$ and 200 μM random oligo (dT) primers, 0.2 μM of each primer, and 2.5 units/ml Taq DNA polymerase (Amersham Biosciences). The reactions were done as follows: 1 min at 95 °C, 35 cycles at 95 °C for 1 min, 55 °C for 1 min, and final extension at 72 °C for 1 min. PCR products were analyzed by electrophoresis on a 2% agarose gel containing ethidium bromide. Densitometrical analysis was performed on Versa-Doc Imaging System (Model 3000, Bio-Rad Laboratories, Hercules, CA, USA).

## 3. Results

### 3.1. NPY content in rat retina and retina cell cultures

Retinas isolated from 9-week-old rats contained 15.1 ± 1.4 fmol NPY/μg protein ($n = 4$). The intracellular content of NPY of retinal cell cultures was 1.4 ± 0.1 fmol NPY/μg protein ($n = 3$, duplicates of three independent cultures). The intracellular content of NPY of rat retina endothelial cell line was below the limit of detection of the NPY assay used (<0.5 pM).

### 3.2. NPY staining in different retina cell types

Labelling NPY and pro-NPY with a highly specific monoclonal antibody, NPY02 (Grouzmann et al., 1992b), revealed the presence of NPY and pro-NPY in rat retinal cells in culture (Fig. 1). Some NPY-positive cells were neurons, since NPY-positive cells were also labelled with a neuronal marker (MAP-2). NPY staining in MAP-2-positive cells was observed mainly in the cell bodies, but some immunoreactivity was also observed in the cell processes (Fig. 1A–C). Some NPY-positive cells were also immunoreactive to GFAP, a marker of astrocytes and Müller cells (Fig. 1D–F). In addition, some NPY-positive cells were microglial cells (Fig. 1G–I). A similar pattern of immunoreactivity was also observed when NPY labelling was assessed with NPY05 antibody (results not shown), another monoclonal antibody (Grouzmann et al., 1992b), which binds the amidated C-terminal part of NPY.

The presence of NPY in Müller cells was confirmed by NPY staining in a cell line of rat Müller cells (Fig. 2B). Retina endothelial cells were also labelled with the NPY02 antibody,

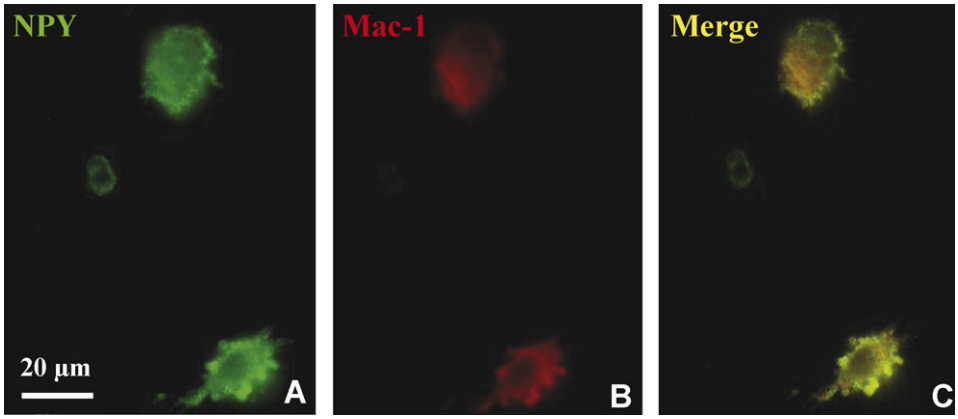


Fig. 3. NPY staining in rat retina microglial cells in culture. NPY was detected (green) with a monoclonal mouse anti-NPY antibody (NPY02; 1:1500). Microglial cells were double stained with the anti-NPY antibody (A) and with an anti-Mac-1 antibody (B). The images are representative of three independent cell cultures. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

and a punctuate labelling throughout the cell was evident, but mostly in the cytosol and in the perinuclear region (Fig. 2A). To corroborate the presence of NPY in microglial cells in retina cells in culture, a primary cell culture of retina microglia cells was used, and as shown in Fig. 3, NPY staining is observed in retina microglial cells in culture.

### 3.3. Detection of mRNA for NPY and NPY receptors by RT-PCR

NPY mRNA was detected in the whole rat retina. The mRNA for NPY was also detected in the retina cells in cultures and in the retina endothelial cell line (Fig. 4A–C). The mRNAs of NPY receptors $Y_1$, $Y_2$, $Y_4$ and $Y_5$ were present in whole rat retina and in the retina cells in culture (Fig. 4A and B). In retina endothelial cells, the mRNAs for NPY $Y_1$, $Y_2$ and $Y_4$ receptors were detected, but the mRNA for $Y_5$ receptor was not detected (Fig. 4C).

### 3.4. NPY release from rat retinal cell cultures

The amount of constitutive amidated-NPY release (10 min in Krebs buffer) from rat retinal cell in culture was below the limit of detection of the NPY assay used. Incubating the cells 10 min with 50 mM KCl the NPY release was 0.5–3.0 pM, which represents a 7.8 ± 4.2% of the intracellular content of NPY ($n = 3$).

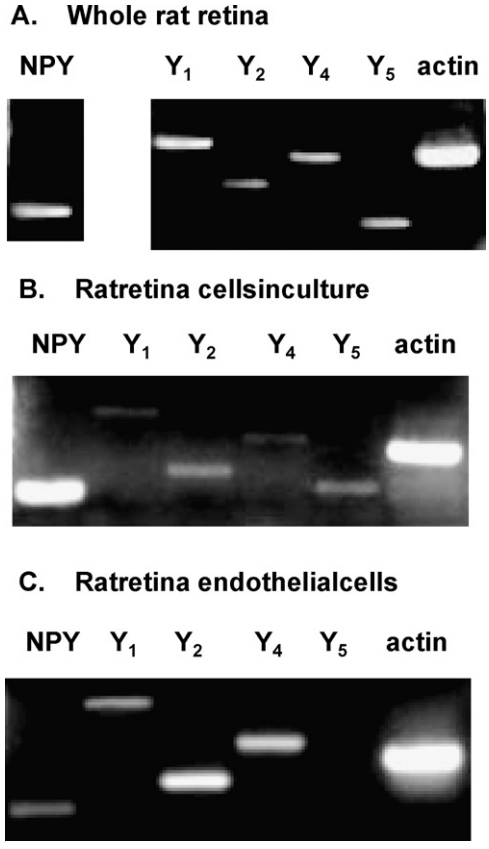


Fig. 4. NPY and NPY receptors mRNAs in whole rat retina (A), rat retina cells in culture (B) and rat retina endothelial cells (C). The mRNA expression of NPY and NPY receptors was assessed by reverse transcriptase-PCR followed by agarose gel electrophoresis. PCR products used (base pairs (bp): NPY, 236 bp; NPY $Y_1$, 616 bp; NPY $Y_2$, 318 bp; NPY $Y_4$, 476 bp; NPY $Y_5$, 248 bp; actin, 427 bp. Each figure is representative of three different retinas or three independent cell cultures.

## 4. Discussion

NPY is present in both mammalian and non-mammalian retinas (Bruun et al., 1986; Hutsler and Chalupa, 1995; Jen et al., 1994; Oh et al., 2002; Tornqvist and Ehinger, 1988). However, its role in retinal physiology and visual processing is not yet elucidated, in part due to lack of information about the retinal cell types that express and release NPY, as well as the expression and localization of NPY receptors. In the present study, using different retina cell cultures, we aimed to identify retinal cell types that express NPY.

Using an ELISA-sandwich assay to quantify the biologically active NPY, amidated-NPY, we verified that the peptide is present in the whole rat retina and in primary retina cells in culture. The presence of NPY in retina cells was demonstrated and confirmed by immunocytochemistry and mRNA expression. The presence of NPY-ir was also observed in rat retina by others (Ferriero and Sagar, 1989; Oh et al., 2002), and also in the retinas of other mammalian species, including human (Jen et al., 1994; Tornqvist and Ehinger, 1988), mouse (Sinclair and Nirenberg, 2001), monkey (Marshak, 1989).

In retina cells in culture, using double immunolabelling, we found that NPY is present in retinal neurons. This observation is in accordance with other studies that described that NPY-ir in the retina is mainly localized in neurons, specially in amacrine cells, but also in ganglion cells (Ammar et al., 1998; Hutsler et al., 1993; Sinclair and Nirenberg, 2001). In rat retina, it was demonstrated that NPY-ir is present in two populations of amacrine cells, one located in the internal nuclear layer and the other in the ganglion cell layer (Oh et al., 2002). These observations suggest that NPY-ir cells participate in multiple circuits, mediating visual information processing, mainly in the inner retina.

Several lines of evidence suggest that the expression of NPY and NPY receptors, in central nervous system, may not be restricted to neurons, but could also extend to glial cells, namely in astrocytes (Barnea et al., 1998; Gimpl et al., 1993; St-Pierre et al., 2000). In fact, we also observed NPY-ir in retina macroglial cells and in microglial cells. These results demonstrate, for the first time that NPY is present not only in retinal neurons, but also in glial cells in the retina. Some studies strongly suggest that retinal macroglia and microglia may be associated with retinal dysfunctions such as vitreoretinopathy, diabetic retinopathy, retinitis pigmentosa and age-related macular degeneration (Gupta et al., 2003; McGillem and Dacheux, 1998; Rungger-Brandle et al., 2000; Soler et al., 2002). Therefore, the presence of NPY in retinal microglia may suggest a putative role of this peptide on inflammatory states observed in those retinal dysfunctions and should be further investigated (Leal et al., 2005; Meleth et al., 2005; Zhou et al., 2005).

In this study, NPY-ir was also detected in cultured rat retina endothelial cells line by immunocytochemistry and mRNA expression. The punctuate labelling was particularly evident in

the cytosol and around the nucleus. NPY-ir was detected in other endothelial cells: in human umbilical-vein endothelial cells (Cai et al., 1993; Silva et al., 2005a) and in human endocardial endothelial cells (Jacques et al., 2003). In this study we detected mRNAs for NPY $Y_1$, $Y_2$ and $Y_4$ receptors but not for $Y_5$ receptor in retina endothelial cells. The presence of mRNA for $Y_1$ and $Y_2$ receptors is consistent with previous studies using HUVECs (Silva et al., 2003; Zukowska-Grojec et al., 1998b). On the contrary, the $Y_4$ receptor mRNA was not detected in HUVECs and the $Y_5$ receptor mRNA was detected only after cytokine treatment (Silva et al., 2003). These differences in NPY receptors mRNA expression between the two types of endothelial cells may influence endothelial cell physiology (Rymaszewski et al., 1992).

Regarding whole retinas and retina cells in culture, in the present work we show that the mRNAs of all known NPY receptors (NPY $Y_1$, $Y_2$, $Y_4$ and $Y_5$) were detected. These results are in accordance to a previous study in whole rat retina (D'Angelo and Brecha, 2004). Also, mRNAs for $Y_1$ receptor and for $Y_2$ receptors were detected in the mouse retina (Yoon et al., 2002). Immunohistochemical studies in rat retina showed that $Y_1$ receptor is present in amacrine cells in the distal inner nuclear layer and also in horizontal cell bodies and their processes in the outer plexiform layer (D'Angelo et al., 2002). Furthermore, in the rat retina there is a difference between the distribution of NPY-ir and $Y_1$ receptor-ir, suggesting that NPY acts in a paracrine manner on cells located far from the site of their release (D'Angelo et al., 2002). The $Y_4$ and $Y_5$ receptors presence in retinal tissue and their cellular localization and physiological role remain to be determined. The presence of functional $Y_2$ receptors in the rat retina, specifically in rod bipolar cells in culture, was indirectly demonstrated by others, who have shown that NPY and two $Y_2$ receptor agonists inhibited the depolarization-induced $Ca^{2+}$ influx into rod bipolar cell axon terminals (D'Angelo and Brecha, 2004).

In the present study, we measure for the first time the release of the biologically active forms of NPY ($NPY_{1-36}$, $NPY_{2-36}$ and $NPY_{3-36}$), evoked by high potassium, in rat retina cells in culture. Other authors, using whole frog retina, also showed that NPY release was evoked by high potassium or by light flashes in a calcium-dependent manner (Bruun et al., 1991).

In conclusion, in the present work, using different rat retina in vitro models (whole retina, rat retinal cell cultures, rat retina microglial cell culture, cell line of rat Müller cells, and cell line of rat retina endothelial cells), we demonstrated that NPY is synthesized and expressed in rat retina in different cells population. A main finding of this study is the fact that NPY staining was detected in retina macroglial (Müller cells) and microglial cells. The role of NPY in retina glial cells should be further investigated and may open new perspectives to study the physiology and the potential pathophysiological function of NPY in the retina.

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