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Expression of the Somatostatin Subtype 2A Receptor in the Rabbit Retina

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Abstract

In the retina, somatostatin influences neuronal activity likely by acting at one or more somatostatin subtype (sst) receptors. Somatostatin and somatostatin-binding sites are distributed predominantly to the inner retina. The present study has investigated the cellular expression of one of the sst receptors, the sst_{2A} receptor isoform, in the rabbit retina. These studies have used a new polyclonal antibody directed to the predicted C-terminus of mouse sst_{2A}(361–369) receptor. Antibody specificity was tested by preadsorption of the primary antibody with a peptide corresponding to sst_{2A}(361–369). sst_{2A} Receptor immunoreactivity was localized mainly to the plasma membrane of rod bipolar cells and to sparsely occurring, wide-field amacrine cells. Immunostaining in rod bipolar cells was strongest in the axon and axon terminals in lamina 5 of the inner plexiform layer (IPL) and was weakest in the cell body and dendrites. Double-labeling experiments using a monoclonal antibody against protein kinase C (PKC; α and β), a rod bipolar cell-selective marker, showed complete colocalization. In horizontal sections of retina, immunostained bipolar cell bodies had a dense distribution, which is in agreement with the reported distribution of rod bipolar cell bodies. Immunoreactive amacrine cell bodies were located at the border of the inner nuclear layer and the IPL, and thin varicose processes ramified mainly in laminae 2 and 4 of the IPL. These observations indicate that somatostatin influences visual information processing in the retina 1) by acting presynaptically on rod bipolar cell axon terminals and b) by influencing the activity of sparsely occurring amacrine cells.

Indexing terms

rod bipolar cells; amacrine cells; immunohistochemistry; neuropeptides; visual system

Somatostatin (or somatotropin-release inhibiting factor; SRIF) a tetradecapeptide that was first isolated from the ovine hypothalamus, is widely distributed throughout the nervous system and peripheral tissues (Brazeau et al., 1973; Epelbaum, 1986). SRIF has a wide variety of biological functions, including the inhibition of endocrine and exocrine secretions and the modulation of transmitter release. SRIF is reported to alter locomotor and behavioral activity and to influence cognitive functions (Epelbaum, 1986; Haroutunian et al., 1987).

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Different experimental approaches indicate that SRIF acts as a transmitter or modulator in the retina. SRIF has been detected in a variety of mammalian retinas, including rat, rabbit, cat, and human, and it has been observed in a number of cell types, including amacrine, interplexiform, and ganglion cells (Sagar and Marshall, 1988; Larsen et al., 1990; White et al., 1990; Rickman et al., 1996). Specific high-affinity SRIF receptor-binding sites have been detected in mammalian retinas, and they have a homogeneous distribution across the inner plexiform layer (IPL; Kossut et al., 1989; Liapakis and Thermos, 1992; Liapakis et al., 1993; Vasilaki et al., 1996). In rabbit retina, SRIF-immunoreactive cells have a broad, asymmetric distribution to the ventral retina and extensive arborization of their cellular processes mainly to laminae 1 and 5 in the IPL in all regions of the retina (Sagar, 1987; Rickman et al., 1996). Pharmacological and electrophysiological studies with an eye cup preparation have shown that SRIF alters the signal-to-noise discharge pattern and the center-surround balance of ganglion cells in the rabbit. Interestingly, these studies also showed that SRIF causes a slow hyperpolarization in rod bipolar cells (Zalutsky and Miller, 1990). In addition, an alteration of SRIF receptor-binding properties has been implicated in a night-blind phenotype and abnormal optokinetic nystagmus in the pearl mutant mouse retina (Balkema et al., 1981; Kossut et al., 1990). Together, these findings suggest that SRIF influences visual information processing in the retina.

Physiological effects of SRIF are diverse. This peptide blocks adenylyl cyclase activity, stimulates tyrosine phosphatase activity, and influences both $K^+$and Ca^{2+} currents (Ikeda and Schofield, 1989; White et al., 1991; Reisine and Bell, 1995). These cellular actions are mediated through seven transmembrane receptors coupled to guanine nucleotide binding proteins. Five distinct somatostatin subtype (sst) receptor genes have been cloned and are designated sst_1 through sst_5 (Bruno et al., 1992; Kluxen et al., 1992; Li et al., 1992; Meyerhof et al., 1992; O'Carroll et al., 1992; Yamada et al., 1992; Yasuda et al., 1992; Hoyer et al., 1995). These receptor genes do not have introns in the coding segments, with the exception of sst_2. The sst_{2A} receptor has two isoforms, sst_{2A} and sst_{2B}, which occur by alternative splicing of the sst_{2A} mRNA (Vanetti et al., 1992). Rodent sst_{2A} and sst_{2B} receptors differ in their length and have unique, predicted C-termini.

The distribution of sst_{2A} receptor immunoreactivity in rabbit retina was determined by using a newly developed polyclonal antibody directed against the C-terminus of mouse sst_{2A}(361–369) receptor (Sternini et al., 1997). A preliminary report of the localization of sst_{2A} receptor immunoreactivity to rod bipolar cells and to sparsely occurring amacrine cells has been published in abstract form (Johnson et al., 1996).

MATERIALS AND METHODS

Tissue preparation

Adult New Zealand albino rabbits were used for this study. They were fed and housed under regular conditions with a 12 hour light-dark schedule. Care and handling of animals were approved by the Animal Research Committee of the VAMC–West Los Angeles in accordance with NIH guidelines.

Rabbits were deeply anesthetized with a mixture of ketamine hydrochloride (35–70 mg/kg, i.m.) and xylazine (5–10 mg/kg, i.m.) followed by sodium pentobarbital (5 ml of 50 mg/ml, i.v.). They were perfused through the heart with 0.1 M phosphate buffered-saline (PBS), pH 7.4. In some cases, the PBS was followed by 2% or 4% paraformaldehyde (PFA) or by 2% or 4% PFA with lysine and periodate (PLP) in 0.1 M phosphate buffer (PB), pH 7.4. The eyes were removed, the anterior segment was dissected, and the posterior eye cup containing the retina was immediately immersed in PFA or PLP in 0.1 M PB. The eye cup was fixed in PFA or PLP in 0.1 M PB from 30 minutes to 2 hours at room temperature, and, in some

cases, the retina was subsequently isolated from the eye cup. The eye cup or isolated retina was then stored overnight in 25% sucrose in 0.1 M PB at 4°C. Sections of the retina were cut perpendicular or parallel to the vitreal surface with a cryostat or a sliding microtome. Cryostat sections were cut at 12–16 μm, mounted onto gelatin-coated slides, air dried, and stored at −20°C. Sliding microtome sections were cut at 25–30 μm and were stored free-floating in 0.1 M PB.

Antibodies

A specific polyclonal antibody (no. 9431) directed against the C-terminus of mouse sst_{2A}(361–369) receptor was used. In most experiments, an affinity-purified antibody was used. Specificity of immunostaining was evaluated by preadsorbing the antibody with 10^{-5} M of the synthetic peptide, sst_{2A}(361–369) receptor. sst_{2A} Receptor immunoreactivity was not present in sections incubated in antibody that was previously preadsorbed with the synthetic peptide. This antibody has been characterized extensively with Western blots and with immunostaining of human embryonic kidney-293 cells transfected with mouse sst_{2A} receptor cDNA (Sternini et al., 1997).

A mouse monoclonal antibody (clone MC5; RPN.536; Amersham, Arlington Heights, IL) directed against the α and β isoforms of protein kinase C (PKC) was used to confirm the identity of rod bipolar cells (Negishi et al., 1988; Wood et al., 1988; Greferath et al., 1990; Vaney et al., 1991; Wässle et al., 1991; Young and Vaney, 1991). A mouse monoclonal antibody directed against tyrosine hydroxylase (TH) was used to identify TH-immunoreactive amacrine cells (Casini and Brecha, 1992).

Immunohistochemistry

Cryostat and free-floating sections were washed in 0.1 M PB and then incubated for 12–36 hours in primary antibody containing 10% normal goat serum and 0.5% Triton X-100 at 4°C. Sections were washed in 0.1 M PB. Sections used for immunofluorescence studies were incubated at a 1:50 or 1:100 dilution in affinity-purified fluorescein isothiocyanate (FITC)-conjugated or tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit or goat anti-mouse immunoglobulins (IgGs; American Qualex, La Mirada, CA, or Jackson Immunoresearch Laboratories, West Grove, PA) for 2 hours at room temperature or overnight at 4°C. Sections used for avidin-biotin peroxidase studies were incubated at a dilution of 1:100 in affinity-purified biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) for 2 hours at room temperature or at 4°C overnight, washed in 0.1 M PB, and then incubated in avidin-biotin-peroxidase complex (ABC) for 2 hours at room temperature. Sections were washed and incubated in 50–100 mg 3,3′-diaminobenzidine tetra-hydrochloride (DAB; Sigma, St. Louis, MO) in 0.1 M Tris, pH 6.5, for 5–10 minutes followed by DAB with 0.03% H_2O_2 for 5–10 minutes. Sections were mounted if free floating and were coverslipped with a glycerol-phosphate or carbonate buffer containing 2% potassium iodide to retard fading for immunofluorescence studies or were coverslipped with Accumount 60 (Baxter, McGaw Park, IL) for ABC studies.

Confocal microscopy

sst_{2A} Receptor immunoreactivity was evaluated in transverse or horizontal sections of retina processed by the immunofluorescence technique. Immunoreactivity was examined with a Zeiss Axiovert (Thornwood, NY) with a Plan Neofluar ×40 1.3 na objective or a PlanApo ×100 1.4 na objective and a Zeiss laser-scanning microscope 410 with a kryptonårgon laser. Optical sections were taken with a z-axis resolution of 1 μm through the immunolabeled cells. Images were collected with a magnification zoom of 1.5×. Figures 8 and 9 were produced by scanning 35-mm Ektachrome color slides with a SprintScan 35/Plus scanner

(Polaroid, Cambridge, MA). Images were processed and labeled by using Adobe Photoshop (version 3.0.5 or 4.0; Adobe Systems, Inc., Mountain View, CA).

In this paper, somatostatin receptor subtype 2A will be designated "sst_{2A}," as recommended by the IUPHAR subcommittee on somatostatin receptors (Hoyer et al., 1995). We also use the term "sst_{2A} receptor immunoreactivity" in place of "sst_{2A} receptor-like immunoreactivity."

RESULTS

Specificity of the antibody

Specific immunoreactivity was eliminated completely by preadsorption of the antibody with a peptide corresponding to sst_{2A}(361–369) receptor (Fig. 1). No differences in sst_{2A} receptor immunoreactivity were observed in tissue that was fixed with 2–4% PFA or PLP.

sst_{2A} Receptor immunoreactivity in rod bipolar cells

sst_{2A} Receptor immunoreactivity was localized mainly to cells that displayed both morphological and positional characteristics of rod bipolar cells (Figs. 1–4). Immunoreactive rod bipolar cells were distinguished from cone bipolar cells by a number of characteristics, including large axonal terminals that only arborized in lamina 5 of the IPL and cell bodies located in the outer part of the inner nuclear layer (INL; Raviola and Raviola, 1967; Famiglietti, 1981; Dacheux and Raviola, 1986; Greferath et al., 1990; Vaney et al., 1991; Wässle and Boycott, 1991; Wässle et al., 1991; Young and Vaney, 1991; Euler and Wässle, 1995).

sst_{2A} Receptor immunoreactivity was strongest in rod bipolar cell axons and terminals, and it was weakest in cell bodies and dendrites (Figs. 1, 2, 4). In transverse sections of retina, the cell body was usually found in the outer portion of the INL, near the outer plexiform layer (OPL; Figs. 1, 2, 4). The weakly stained dendrites formed a very fine plexus of processes in the OPL. The axon descended through the INL and the distal IPL, where it terminated in the IPL, adjacent to the ganglion cell layer (GCL), as club-or knob-like processes. The predominant localization of sst_{2A} receptor immunoreactivity at or near the cell surface was observed in 1-μm optical sections through the cell body, axon, and axon terminal (Fig. 2).

In horizontal sections of retina, sst_{2A} receptor-immunostained cell bodies were found to have a dense distribution, which is in agreement with the reported distribution of PKC-immunoreactive cells (Fig. 3; Vaney et al., 1991). Cell bodies measured 6 ± 0.5 μm (mean ± S.D.; n = 8) in diameter. sst_{2A} Receptor-immunostained axon terminals in the IPL measured 2 ± 0.41 μm (mean ± S.D.; n = 13) in diameter, which is similar to the size of these terminals as reported on the basis of Golgi and intracellular labeling preparations (Dacheux and Raviola, 1986).

Double-labeling experiments with PKC antibodies were used to further characterize the sst_{2A} receptor-immunoreactive pattern. A monoclonal antibody against PKC specifically labels rod bipolar cells (Negishi et al., 1988; Wood et al., 1988; Greferath et al., 1990; Vaney et al., 1991; Wässle et al., 1991; Young and Vaney, 1991). sst_{2A} Receptor and PKC immunoreactivities were expressed in the same bipolar cell bodies, axons, and axonal terminals (Fig. 4). Interestingly, rarely occurring PKC-immunoreactive amacrine cells (Negishi et al., 1988) did not express sst_{2A} receptor immunoreactivity.

sst_{2A} Receptor immunoreactivity in amacrine cells

sst_{2A} Receptor immunostaining was also localized to infrequently occurring amacrine cell bodies located in the proximal INL (Figs. 5–9). Immunostained cell bodies were always located at the border of the INL and the IPL. In addition, there were thin, varicose processes in laminae 2 and 4 of the IPL in all retinal regions (Figs. 5, 8, 9). In transverse sections, these processes were not continuous in these laminae. In horizontal sections, the processes were wide spread and were observed at different levels in the IPL, corresponding best to laminae 2 and 4. We did not attempt to evaluate the extent of these processes to determine the overall size of individual sst_{2A} receptor-immunoreactive amacrine cells. In horizontal sections of retina, sst_{2A} receptor-immunoreactive amacrine cell bodies had a diameter of 19 ± 1.5 μm (mean ± S.D.; n = 3). Strong immunostaining outlined these cell bodies. The close association of sst_{2A} receptor immunoreactivity with amacrine cell plasma membrane was further established in 1-μm optical sections by using confocal microscopy (Fig. 7).

Double-labeling experiments with sst_{2A} receptor and TH antibodies were performed to further characterize the distribution of sst_{2A} receptor-immunoreactive processes in the IPL. TH has been localized to wide-field amacrine cells in the rabbit retina, which give rise to processes that ramify mainly in lamina 1 as well as in lamina 3 and 5 of the IPL (Tauchi et al., 1990; Brecha et al., 1991; Casini and Brecha, 1992). There was no colocalization of TH and sst_{2A} receptor immunostaining (Figs. 8, 9). The TH-immunoreactive processes in lamina 1 were distributed between the INL and the sst_{2A} receptor-immunoreactive processes in lamina 2. In addition, the TH-immunoreactive processes in lamina 3 were between the sst_{2A} receptor-immunoreactive processes in laminae 2 and 4. Finally, some sst_{2A} receptor-immunoreactive processes were observed in laminae 1 and 3. They were visualized most clearly by using confocal microscopy in 1-μm optical sections (Fig. 2). These immunoreactive processes are likely to be from amacrine processes that transverse these laminae to ramify in laminae 2 and 4 of the IPL (Figs. 5, 7–9).

DISCUSSION

These investigations show that the immunoreactive SRIF receptor isoform, sst_{2A}, is expressed by rod bipolar and amacrine cells of the rabbit retina (Fig. 10). sst_{2A} Receptor immunoreactivity was determined by using a new polyclonal antibody directed against a synthetic peptide corresponding to the C-terminus of the mouse sst_{2A} receptor. The predicted peptide sequence used for antibody production is identical to that of the C-terminus of the rat, mouse, and human sst_{2A} receptor (Kluxen et al., 1992; Yamada et al., 1992). sst_{2A} Receptor immunoreactivity was localized predominantly to rod bipolar cell axons and terminals, and double-labeling experiments showed complete colocalization of sst_{2A} receptor and PKC (α and β) immunoreactivity in these cells. sst_{2A} Receptor immunoreactivity was also observed in large, sparsely occurring, wide-field amacrine cells that were located at the border of the INL and IPL. They gave rise to thin, varicose processes that ramify predominantly in laminae 2 and 4 of the IPL. The sst_{2A} receptor-immunoreactive amacrine cells were not PKC-immunoreactive and appeared to differ in their morphology and distribution from previously reported amacrine cell types (Vaney et al., 1988; Vaney, 1990).

Anatomical, biochemical, and electrophysiological studies indicate a role for SRIF as a neuromodulator in the rabbit retina (Tornqvist et al., 1982; Sagar et al., 1986; Sagar, 1987; Rickman and Brecha, 1989; Zalutsky and Miller, 1990; Liapakis and Thermos, 1992; Liapakis et al., 1993; Rickman et al., 1996; Vasilaki et al., 1996). SRIF immunoreactivity has been localized to a sparse population of wide-field amacrine cells that are located predominantly in the GCL of the ventral retina (Fig. 10; Tornqvist et al., 1982; Sagar et al., 1986; Sagar, 1987; Rickman and Brecha, 1989; Rickman et al., 1996). The extensive

distribution of the processes of these cells to all retinal regions indicates that these cells may have a modulatory role in the retina and may influence a number of cell types, including amacrine and bipolar cells (Masland, 1988; Zalutsky and Miller, 1990; Rickman et al., 1996). Electrophysiology studies also support a role for SRIF as a modulator in rabbit retina (Zalutsky and Miller, 1990). For example, in rabbit retinal eye-cup preparations, SRIF changes the signal-to-noise discharge pattern and the center-surround balance of ganglion cells. These changes in ganglion cell properties are thought to be due to SRIF's action on multiple levels of the retinal circuitry, including the modulation of third-order neurons (Zalutsky and Miller, 1990). The observed expression of sst_{2A} receptors by rod bipolar cells and amacrine cells extends these earlier studies and suggests that SRIF can influence neuronal activity in the inner retina through the sst_{2A} receptor expressed by rod bipolar cells and amacrine cells.

sst_{2A} Receptor is expressed by rod bipolar cells

Most sst_{2A} receptor-immunoreactive cells are rod bipolar cells, based on their appearance, size, and distribution and on colocalization of the sst_{2A} receptor and PKC immunoreactivities (Raviola and Raviola, 1967; Famiglietti, 1981; Dacheux and Raviola, 1986; Negishi et al., 1988; Wood et al., 1988; Greferath et al., 1990; Vaney et al., 1991; Wässle and Boycott, 1991; Wässle et al., 1991; Young and Vaney, 1991; Euler and Wässle, 1995). Cone bipolar cells did not appear to have sst_{2A} receptor immunoreactivity. Interestingly, sst_{2A} receptor immunoreactivity was not distributed uniformly in rod bipolar cells. Immunostaining was strongest in the axon and terminal and was weakest in the cell body and dendrites. These observations suggest that sst_{2A} receptor activation by SRIF may influence the excitability of the rod bipolar axonal terminals. SRIF is not likely to influence glycine or γ-aminobutyric acid ionotrophic receptors in isolated rabbit rod bipolar cells (Gillette and Dacheux, 1995); however, SRIF could influence glutamate receptors (Peng et al., 1995; Lanneau et al., 1996) or directly influence ion channels.

SRIF could directly mediate presynaptic transmission by the regulation of ion channels via the sst_{2A} receptor. Pharmacology studies report that the sst_{2A} receptor likely regulates K^+ and Ca^{2+} channels through interactions with G-proteins in neurons (Reisine and Bell, 1995). Furthermore, the application of selective sst_{2A} receptor agonists to freshly dissociated amygdaloid neurons causes an inhibition of high-voltage-activated N- and P/Q-type calcium channels, probably through the sst_{2A} receptor (Viana and Hille, 1996). Electrophysiology studies of rabbit retina report that SRIF causes a slow hyperpolarization and amplification of the light response in rod bipolar cells (Zalutsky and Miller, 1990). These authors proposed that this effect was a result of SRIF closing cation channels in other cell types (Zalutsky and Miller, 1990). Because the sst_{2A} receptor is known to modulate cation channels (Ikeda and Schofield, 1989; White et al., 1991; Reisine and Bell, 1995), this action suggests a mechanism by which SRIF may influence one or more of the channels in rod bipolar cells. Interestingly, in rod bipolar cells isolated from goldfish retina, SRIF also causes a closure of cation channels, which results in suppression of L-type Ca^{2+} current (Ayoub and Matthews, 1992). Perhaps SRIF acts by similar mechanisms in both goldfish and rabbit rod bipolar cells.

These studies show a greater density of sst_{2A} receptor immunoreactivity compared with the number and distribution of SRIF-immunoreactive processes and cells (Fig. 10; Sagar et al., 1986; Sagar, 1987; Rickman and Brecha, 1989; Rickman et al., 1996). In addition, SRIF-immunoreactive processes in lamina 5 are very sparse, with few synaptic profiles in relation to the large number of rod bipolar terminals in this lamina (Rickman et al., 1996). Assuming that the sst_{2A} receptors are functional, then there is a mismatch (for review, see Herkenham, 1987) between the distribution of SRIF and sst_{2A} receptor immunoreactivities in the rabbit retina. Indeed, this mismatch between the localization of peptide and receptor indicates that

SRIF likely diffuses over many micrometers before interacting with the sst_{2A} receptor on rod bipolar cell axonal terminals, thus suggesting a paracrine action for SRIF in the retina.

sst_{2A} Receptor is expressed by amacrine cells

sst_{2A} Receptor immunoreactivity was also observed in cells that are wide-field amacrine cells based on their appearance, size, and distribution. The immunoreactive amacrine cells appear to be unique from previously reported rabbit amacrine cells identified by histochemical and other techniques. Although they do have some resemblance to the wide-field amacrine cell types that have been identified by using reduced silver stains (Vaney et al., 1988), they are not likely to be the same cell type, that is, the reduced, silver-stained amacrine cells differ in their morphology compared with the sst_{2A} receptor-expressing amacrine cells in the distribution of their processes in the IPL (Vaney et al., 1988).

The function of sst_{2A} receptor amacrine cells is unknown, but the infrequently occurring cells bodies and the apparent wide arborization of their processes indicate that these cells are not likely to participate in the direct flow of information through the retina (Masland, 1988; Tauchi et al., 1990; Rickman et al., 1996). The slow onset and long-lasting effects of SRIF shown in the electrophysiology experiments with rabbit retina (Zalutsky and Miller, 1990), together with the morphology of the sst_{2A} receptor amacrine cells, indicate that these cells are likely to have broad or global influences in the inner retina (Masland, 1988).

Multiple sst receptor subtypes may be present in the rabbit retina like in other tissue, such as rat brain (Señaris et al., 1994; Viollet et al., 1995). Assuming the presence of multiple sst receptors in the rabbit retina, these receptors may be expressed by other cell types, which suggests that SRIF may act on other cell types in the rabbit retina. Further studies investigating the sites of expression and ultrastructural organization of the sst receptors will further elucidate the role of SRIF in influencing retinal function.

In conclusion, the pattern of sst_{2A} receptor expression suggests that SRIF modulates visual information processing in the retina both at rod bipolar cells and at sparsely occurring amacrine cells. SRIF could influence presynaptic glutamate transmission via the sst_{2A} receptor on rod bipolar cell axonal terminals. In addition, SRIF could also influence the sparsely occurring wide-field amacrine cells, which are hypothesized to have a modulatory role in visual processing in the inner retina.

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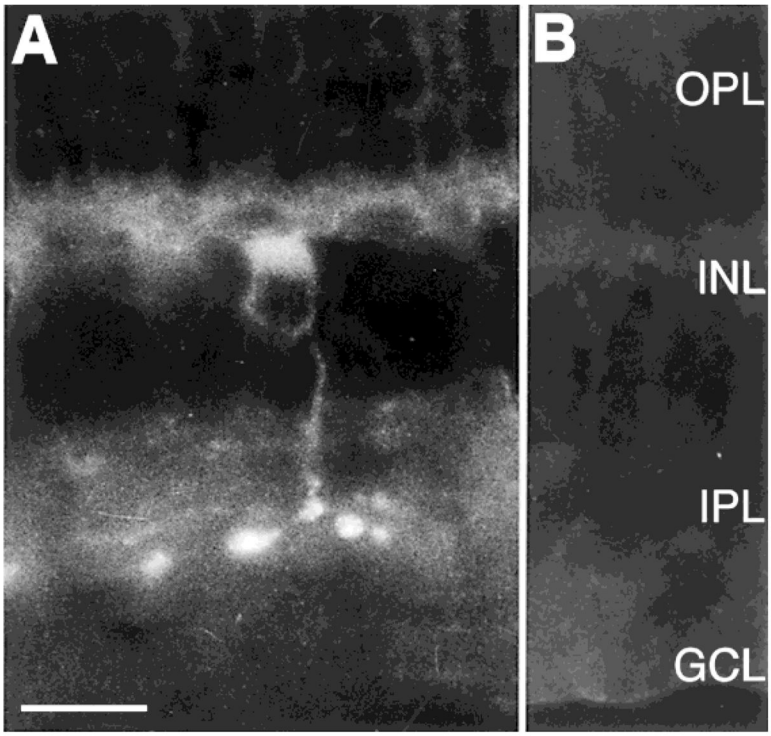

Fig. 1.

Localization of somatostatin subtype 2A (sst_{2A}) receptor immunoreactivity in the rabbit retina. **A:** The sst_{2A} receptor immunostaining was prominent in rod bipolar cells. **B:** Lack of sst_{2A} receptor immunostaining in a control section incubated with the sst_{2A} receptor antibody preadsorbed with 10^{-5} M sst_{2A} (361–369). OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 15 μm.

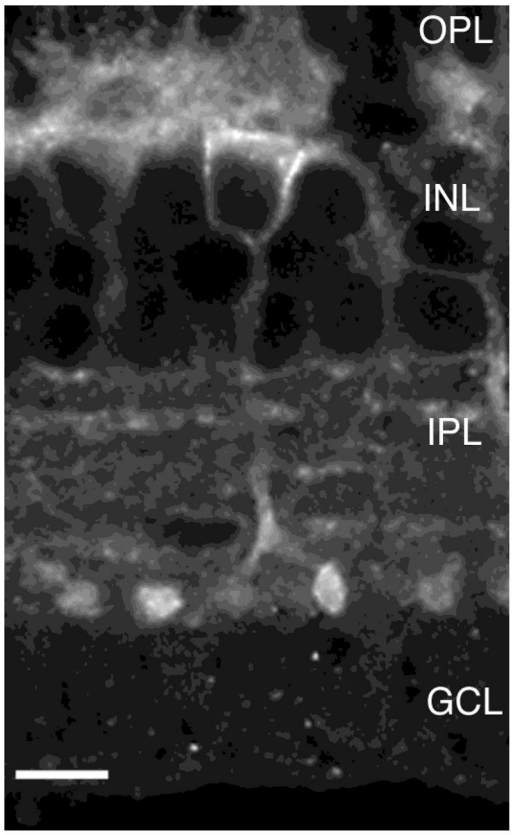

Fig. 2.

Confocal image of sst_{2A} receptor-immunoreactive rod bipolar cell in a transverse section of retina. sst_{2A} Receptor immunoreactivity was closely associated with the plasma membrane of rod bipolar cells. The axon descended into the inner plexiform layer (IPL), where it terminated with a few large, club-like processes in lamina 5 of the IPL. sst_{2A} Receptor immunoreactivity was also associated with thin processes that ramified in laminae 2 and 4 of the IPL. These processes originated from sparsely occurring amacrine cells. OPL, outer plexiform layer; INL, inner nuclear layer; GCL, ganglion cell layer. Z axis = 1 μm. Scale bar = 7.5 μm.

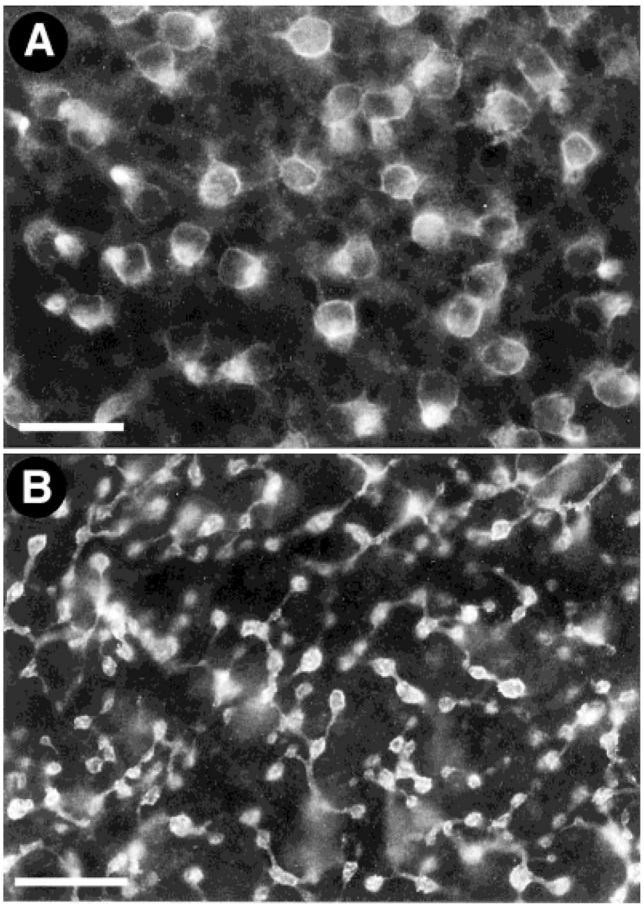

Fig. 3.

The sst_{2A} receptor-immunostained cell bodies and axon terminals were found in the distal inner nuclear layer (INL) and proximal INL, respectively. Horizontal sections through the INL (**A**) showing sst_{2A} receptor-immunostained cell bodies and through the inner plexiform layer (IPL; **B**) showing sst_{2A} receptor-immunostained axon terminals. Scale bars = 25 μm in A, 15 μm in B.

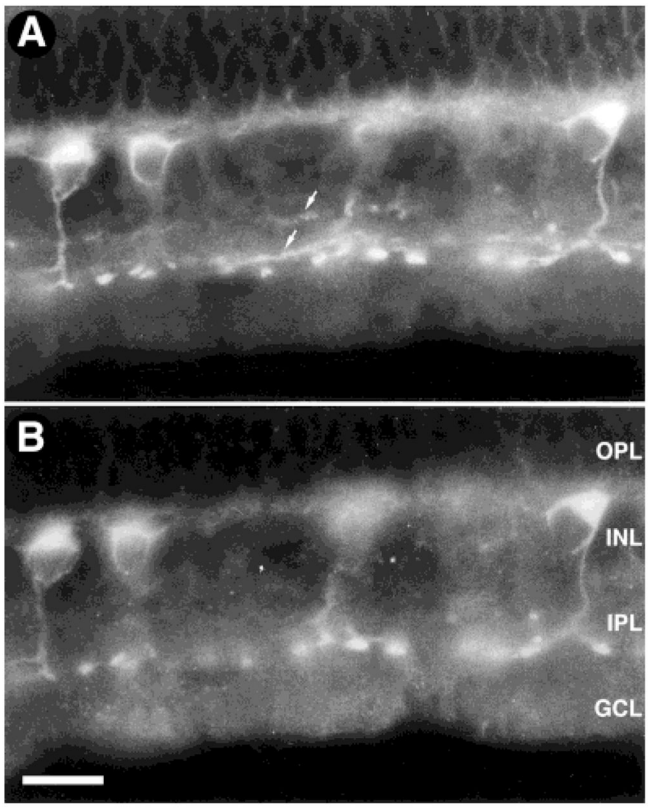

Fig. 4.

Coexpression of sst_{2A} receptor (**A**) and protein kinase C (PKC; **B**) immunoreactivities in rod bipolar cells. The sst_{2A} receptor showed complete coexpression with the rod bipolar, cell-selective marker PKC, further confirming that the sst_{2A} receptor is expressed by rod bipolar cells. OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 15 μm.

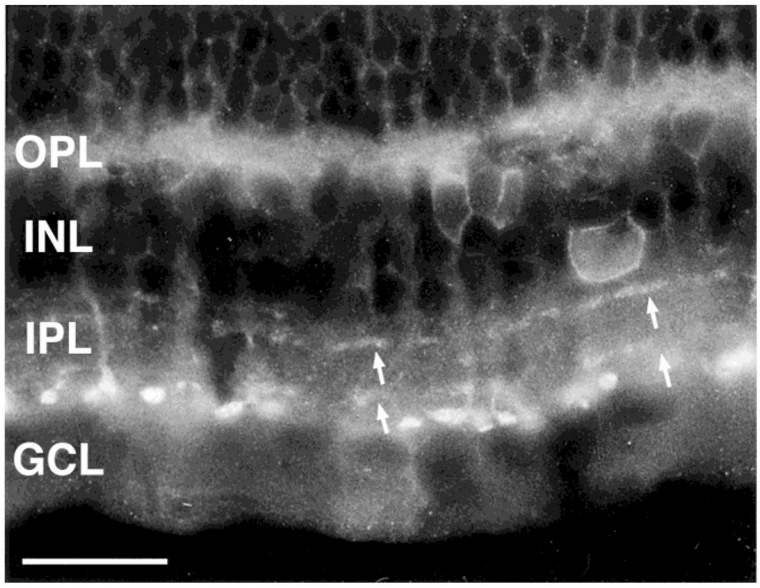

Fig. 5.

The sst_{2A} receptor-immunoreactive amacrine cells were sparsely occurring. Immunoreactive cell bodies were located at the border of the inner nuclear layer (INL) and the inner plexiform layer (IPL). They were likely to be multistratified and gave rise to the processes in laminae 2 and 4 of the IPL. In transverse sections, these processes in laminae 2 and 4 were not continuous. OPL, outer plexiform layer; GCL, ganglion cell layer. Scale bar = 25 μm.

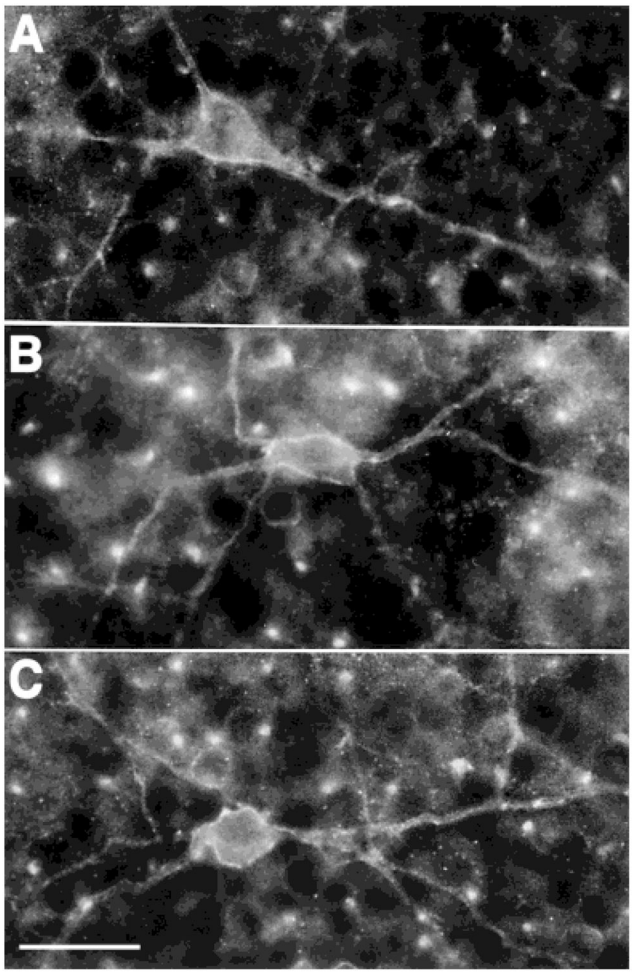

Fig. 6.

A–C: The sst_{2A} receptor-immunoreactive amacrine cells in a horizontal section of the retina. Strong immunostaining outlined these cell bodies and their main processes. Scale bar = 25 μm.

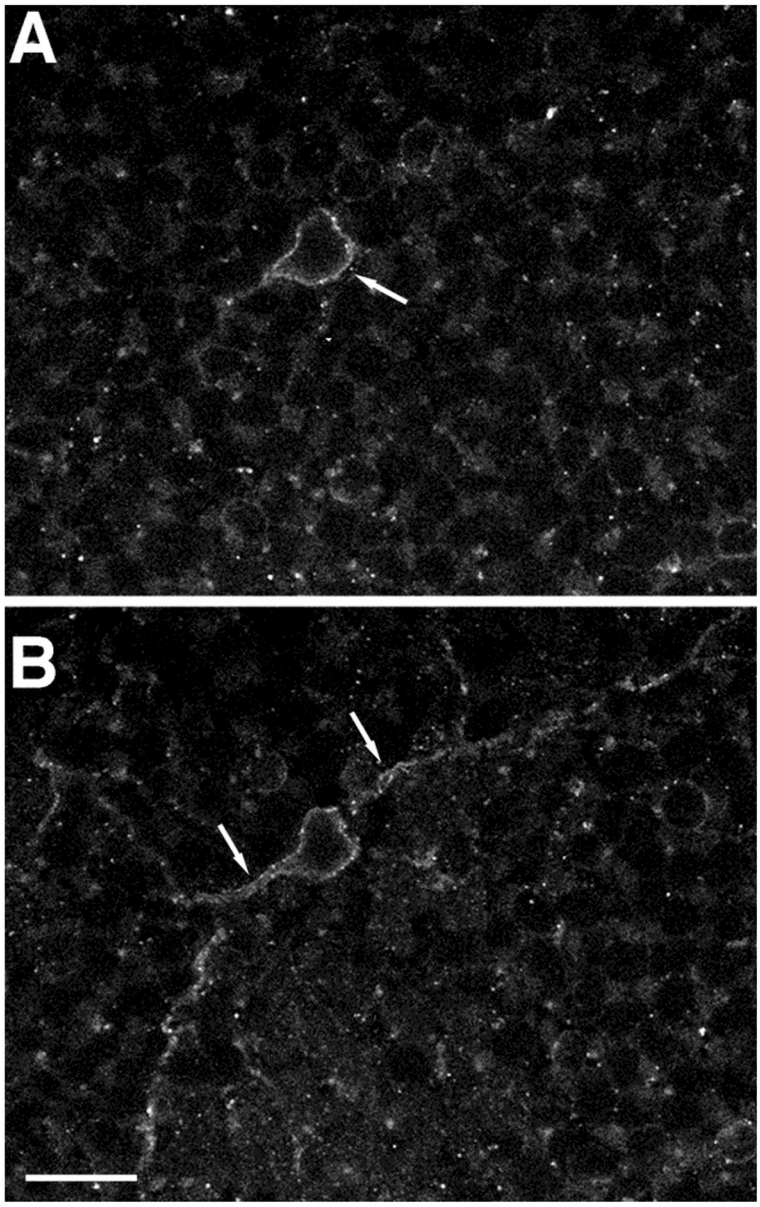

Fig. 7.

Confocal image showing the localization of sst_{2A} receptor immunoreactivity closely associated with the plasma membrane of amacrine cells. Two different planes of focus through the same cell. Arrows indicate sst_{za} immunoreactivity associated with the plasma membrane in the cell body (**A**) and the cell body and processes (**B**). Z axis = 1 μm. Scale bar = 12.5 μm.

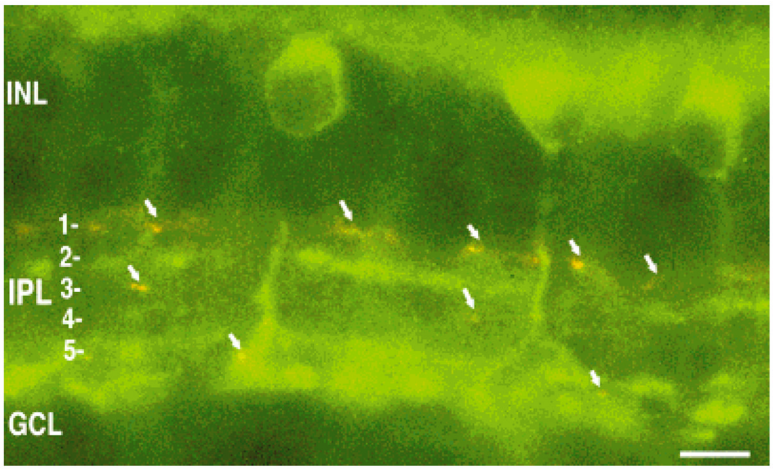

Fig. 8.

Lack of colocalization of sst_{2A} receptor and tyrosine hydroxylase (TH) immunoreactivities. Composite image of sst_{2A} receptor (green) and TH (red) immunoreactivities in a transverse section of retina. Arrows indicate TH-immunoreactive processes. INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 10 μm.

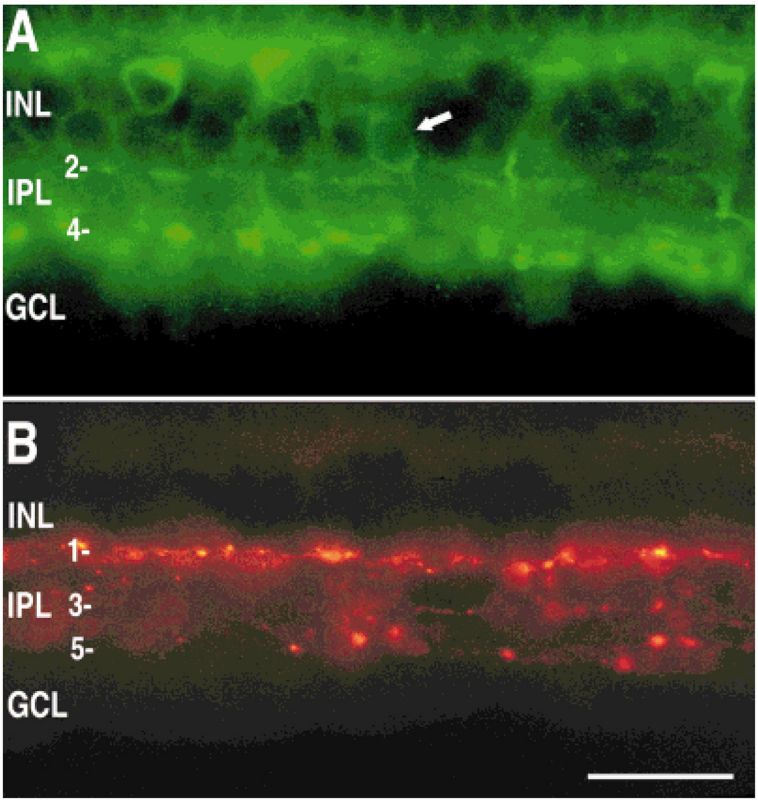

Fig. 9.

Localization of sst_{2A} receptor (**A**) and TH (**B**) immunoreactivities in the same section of rabbit retina. A: The sst_{2A} receptor containing rod bipolar cells and amacrine cells. Note the amacrine cell body (arrow) and processes in laminae 2 and 4. B: TH-immunoreactive processes were localized to laminae 1, 3, and 5 of the IPL. INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 25 μm.

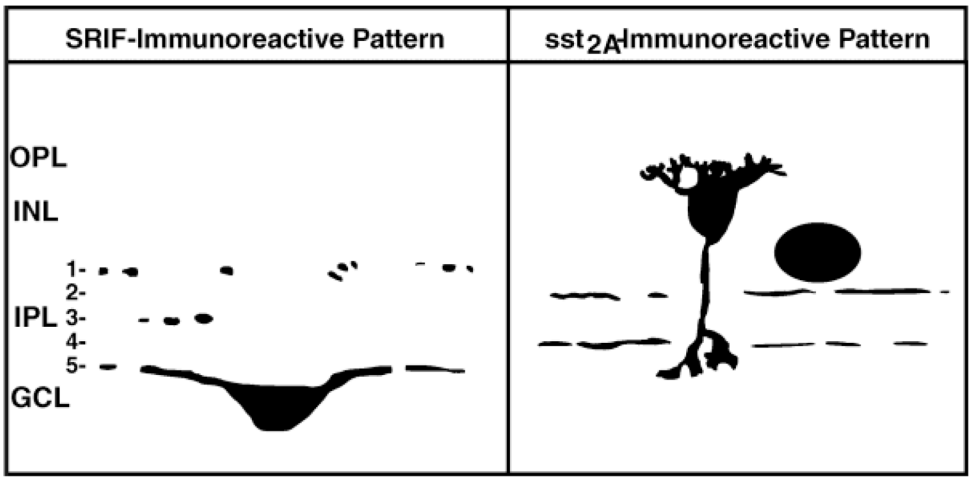

Fig. 10.

Schematic diagram illustrating the distribution of somatostatin and sst_{2A} receptor immunoreactivities in rabbit retina. Images of the rod bipolar cell and the somatostatin-immunoreactive amacrine cell were adapted from Rickman et al. (1996) and Gillette and Dacheux (1995). SRIF, somatotropin-release inhibiting factor; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.