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Functional mapping of somatostatin receptors in the retina: a review

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Abstract

The peptide somatostatin is one of many neuroactive agents that influence retinal physiology. It is synthesized primarily in a subclass of amacrine cells and believed to function as a neurotransmitter, neuromodulator or trophic factor. The cloning of the somatostatin receptors (sst_{1-5}) in the early nineties provided the appropriate tools for the study of sst_s in many tissues, including the retina. In this review, emphasis is given to recent studies that have provided significant information on the functional role of somatostatin in retinal circuitry and the retinal pigment epithelium. The important role of somatostatin in retinal disease therapeutics is also discussed.

Keywords: Neural retina; Retinal pigment epithelium; Somatostatin receptors; NADPH-diaphorase; Tyrosine hydroxylase

1. Introduction

The neuropeptide somatostatin (somatotropin release inhibitory factor, SRIF) is a cyclic tetradecapeptide, which is widely distributed in the peripheral and central nervous system (Brazeau et al., 1973; Epelbaum, 1986). It mediates a diverse number of physiological actions by interacting with specific receptors in the plasma membrane (Thermos, He, Wang, Margolis, & Reisine, 1989; Thermos & Reisine, 1988). Five SRIF receptor subtypes have been cloned, namely sst_{1-5} (Bruno, Xu, Song, & Berelowitz, 1992; Meyerhof, Wylfsen, Schonrock, Fehr, & Richter, 1992; O'Carroll, Lolait, Konig, & Mahan, 1992; Yamada et al., 1992). The sst_2 has been identified in mouse and rat to exist as two-splice variants sst_{2A} and sst_{2B} (Schindler et al., 1998; Vanetti, Kouba, Wang, Vogt, & Hollt, 1992). These receptors are expressed differentially in different tissues, coupled to different G-proteins and modulate the actions of diverse second messengers (Florio & Schettini, 1996; Hoyer et al., 1995; Patel & Srikant, 1994; Reisine & Bell, 1995; Tannenbaum & Epelbaum, 2000).

Somatostatin is one of many neuroactive substances that influence retinal physiology. Its presence has been detected with mRNA, radioimmunoassay, and immunohistochemistry techniques (Akopian, Johnson, Gabriel, Brecha, & Witkovsky, 2000; Larsen, Bersani, Olcese, Holst, & Moller, 1990; Rickman, Blanks, & Brecha, 1996; Rorstad, Brownstein, & Martin, 1979; Rorstad, Senternman, Hoyte, & Martin, 1980; Sagar, Marshall, & Landis, 1985; Sagar, Rorstad, Landis, Arnold, & Martin, 1982; Sagar & Marshall, 1988; Sagar, 1987; Shapiro, Kronheim, & Pinistone, 1979; Spira, Schimizu, & Rorstad, 1984; Tornqvist, Uddman, Sundler, & Ehinger, 1982; White, Chalupa, Johnson, & Brecha, 1990; Yamada et al., 1980; Yamaguchi, Gaur, Spira, & Turner, 1990). SRIF is localized primarily in wide-field amacrine cells with processes that ramify in the inner plexiform layer (IPL), in cells of the ganglion cell layer (GCL) and in neurons in the inner nuclear layer (INL), some of which are probably interplexiform cells (Akopian et al., 2000; Larsen et al., 1990; Rickman et al., 1996; Sagar et al., 1985; Sagar & Marshall, 1988; Sagar, 1987; Tornqvist et al., 1982; White & Chalupa, 1991; White et al., 1990).

Electrophysiological studies have supported that SRIF may function as a neurotransmitter, neuromodulator or trophic factor (Adolph, 1989; Akopian et al., 2000; Petrucci, Resta, Fieni, Bigiani, & Bagnoli, 2001;

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Zalutsky & Miller, 1990). SRIF was shown to enhance the spontaneous firing and light-evoked activity of ganglion cell activity in turtle retina (Adolph, 1989). In an extensive electrophysiological study Zalutsky and Miller (1990) also detected a general excitation on rat ganglion cells, in addition to an increase in the "signal-to-noise ratio" defined as the ratio of light-evoked to spontaneous spiking, and a shift in center-surround balance towards a more dominant center. The authors suggested that SRIF acts at different neurons of the retinal circuitry (direct action on ganglion and amacrine cells) to produce the above mentioned actions. In a more recent investigation, Akopian et al. (2000) presented evidence to support somatostatin's modulation of voltage-gated K^+ and Ca^{++} currents in photoreceptors, while Petrucci et al. (2001) supported somatostatin's modulation of potassium current and calcium influx in isolated rod bipolar cells of the rabbit.

The purpose of this review is to summarize experiments that have been performed during the last decade and have shed some light on the functional role of somatostatin in retinal circuitry and its possible therapeutic applications in retinal disease. In particular, emphasis is given to the immunohistochemistry studies performed over the last five years, the results of which provided significant information on the localization of the somatostatin receptor subtypes in retinal neurons and subsequently their function.

2. Somatostatin receptors in the retina

Prior to the cloning of the somatostatin receptors, pharmacological studies had shown the presence of somatostatin binding sites in the retina. Radioligand binding experiments were performed in the retina initially in the mid eighties. Specific binding sites for [^{125}I]Tyr^{11} somatostatin-14 were characterized in the cytosol fraction of the pig retina (Colas, Arilla, & Prieto, 1986). Subsequent studies employing either autoradiography or membrane binding techniques and the radioligands [^{125}I]Tyr^{11} somatostatin-14, [^{125}I]Tyr^0, DTrp^8]somatostatin-14, [^{125}I] [Leu8, D-Trp22, Tyr25]somatostatin-28, and [^{125}I] SMS204-090 provided evidence of high affinity binding sites for somatostatin in mouse (Kossut, Aldrich, Yamada, & Pinto, 1990; Kossut, Yamada, Aldrich, & Pinto, 1989), ovine (Colas, Valencia, Prieto, & Arilla, 1992) and rabbit retinas (Liapakis, Politou, & Thermos, 1993; Liapakis & Thermos, 1992; Vasilaki, Hatzilaris, Liapakis, Georgoussi, & Thermos, 1996), as well as in the rat retina during development (Bodenant, Leroux, Gonzalez, & Vaudry, 1991; Ferriero, 1992). Pharmacological and biochemical studies in the ovine and rabbit retinas presented evidence showing that the somatostatin receptors are coupled to G-proteins and influence adenylate cyclase activity (Colas et al., 1992; Liapakis & Thermos, 1992).

The cloning of five somatostatin receptor subtypes (Bruno et al., 1992; Meyerhof et al., 1992; O'Carroll et al., 1992; Yamada et al., 1992) created new avenues for the study of somatostatin receptors and their physiological function in many tissues including the retina. Mori, Ahara, and Shimizu (1997) performed the first reverse transcription-polymerase chain reaction (RT-PCR) studies in rat ocular tissues and reported the presence of all ssts in the retina with the sst_2 subtype being the predominant. Johnson, Wu, Wong, Walsh, and Brecha (1999) subsequently examined the presence of mRNA of the two-splice variants of the sst_2 receptor in rat retina and reported the expression of sst_{2A} but not the sst_{2B} receptors. In the rabbit retina, sst_1 was reported to be the predominant subtype, but moderate to low levels of the other receptor subtypes were also expressed (Cristiani et al., 2000). Most recently, all ssts were detected in mouse retina with high levels of sst_2 and sst_4 mRNA and moderate levels of sst_1 and sst_5 mRNA (Cristiani, Petrucci, Dal Monte, & Bagnoli, 2002). In a review by Van Hagen et al. (2000), sst_1, sst_{2A}, and sst_3 mRNAs were reported to be also present in human retina. In agreement with these data, sst_1, sst_{2A}, and sst_3 mRNAs were also detected in the posterior pole, which included the retina, in normal human eyes (Lambooij et al., 2000). In addition, a study by Klisovic et al. (2001) presented evidence to support the expression of genes for all receptor subtypes (sst_{1-5}) in normal human retina, with the sst_3 and sst_5 genes being expressed only in the retina, among the ocular tissues examined.

While the RT-PCR studies performed in the different species are limited, one can detect a consensus in the expression of all subtypes in the mouse, rat, and rabbit retinas with differences observed in the quantity of each receptor (Cristiani et al., 2000, 2002; Mori et al., 1997). In the human retina, there is agreement with respect to the expression of sst_1, sst_2/sst_{2A}, and sst_3 subtypes. The discrepancy in the presence or absence of the sst_4 and sst_5 receptors may be due to the different retinal preparations employed, as well as the primer sequences used for the RT-PCR studies (Klisovic et al., 2001; Lambooij et al., 2000).

With the recent development of specific antibodies for each receptor subtype, immunohistochemistry studies have given important information on the identity and localization of each receptor in retinal cells of different species.

2.1. sst_1

Helboe and Moller (1999) first reported the localization of sst_1 receptors in rat retina, using a rabbit polyclonal antiserum raised against fragments of the C-terminal part of the human sst_1 (Helboe, Moller,

Norregaard, Schiodt, & Stidsen, 1997). sst_1 immunoreactivity was found to be present in SRIF expressing amacrine cells located in the INL, in displaced amacrine cells in the GCL and in a small number of ganglion cells.

Cristiani et al. (2000) employed the sst_1 antibody produced by Helboe et al. (1997) and showed sst_1 immunoreactivity in amacrine cells (non-tyrosine hydroxylase (TH)- and TH-immunoreactive), displaced amacrines and in the GCL of the ventral rabbit retina. Helboe and Moller (1999) did not observe sst_1 immunoreactivity on TH-amacrine cells or their processes in the rat retina. In agreement with the latter study, we did not detect sst_1 immunoreactivity in TH or ChAT immunoreactive processes located in the sublamina S1 of the IPL. We employed a rabbit antiserum raised against a peptide fragment (374–391) of the $rsst_1$ receptor subtype (Schulz, Handel, Schreff, Schmidt, & Hollt, 2000) and detected sst_1 immunoreactivity in cell processes lengthening throughout the IPL in sublamina S1, in blood vessels of the inner retina, and the retinal pigment epithelium (RPE) of the rat (Vasilaki, Mouratidou, Schulz, & Thermos, 2002). sst_1 immunoreactivity was reported in human retina to be localized in retinal ganglion cells, the INL and the RPE (Van Hagen et al., 2000). In an attempt to examine the somatostatin receptor gene expression and localization in human ocular tissue (Klisovic et al., 2001), reported sst_1 immunoreactivity in membranes of outer and inner segments of rods and cones, and individual cells in the ONL, INL and GCL. sst_1 immunostain was also present in retinal blood vessels, in cultured RPE cells, and human RPE tissue. The antibody employed in the latter study was a rabbit polyclonal antiserum raised against a peptide antigen made of the N-terminal 57 amino acids of $hsst_1$ (Albers et al., 2000).

2.2. sst_2

A pharmacological study performed by Vasilaki et al. (1996) suggested the presence of sst_2 receptors in rabbit retina, yet it was Johnson, Wong, Walsh, and Brecha (1996, 1998) who first showed the expression of the sst_{2A} receptor in neurons of the rabbit retina. These investigators employed a polyclonal antibody raised against the C-terminus of mouse sst_{2A} (361–369) and indicated sst_{2A} receptor localization mainly in the plasma membrane of rod bipolar cells and to sparsely occurring wide-field amacrine cells of the rabbit retina. Immunostaining in the rod bipolar cells was strongest in the axon and axon terminals in lamina 5 of the IPL and was weakest in the cell body and dendrites. Immunoreactive amacrine cell bodies were located at the border of the INL and the IPL. Also, in rabbit retina, Petrucci et al. (2001) identified sst_{2A} receptors in isolated rod bipolar cells, while Fontanesi, Gargini, and Bagnoli (2000) reported the postnatal development of sst_{2A} expression in rod bipolar cells and amacrine cells, some of which were TH positive. The antibodies employed in these last two studies were the same, a sheep polyclonal antibody directed against the amino acid sequence 347–366 of the carboxy terminus of rat sst_{2A}. This peptide sequence is identical in rat, human and mouse sst_{2A} receptors (Schindler, Sellers, Humphrey, & Emson, 1997).

In the rat retina, sst_{2A} immunoreactivity was localized in the outer retina to cone photoreceptors, horizontal cells and rod and cone bipolar cells, while in the inner retina to TH-positive amacrine cells of medium to large size (Johnson et al., 1999). The antibody employed was the same as in the rabbit studies by Johnson et al. (1998). Helboe and Moller (1999) employing a rabbit polyclonal antibody raised against the C-terminal parts of the human sst_2 noted immunoreactivity in large amacrine cells of the INL that also contained TH, in inner segments of cone photoreceptors and in Muller cell fibers. sst_2 immunoreactivity was not detected in horizontal cells nor rod bipolar cells. Vasilaki, Gardette, Epelbaum, and Thermos (2001) reported sst_{2A} immunoreactivity in rod bipolar cells of the rat and rabbit retinas, using a rabbit polyclonal antibody raised against the amino acid sequence 355–369 of the $rsst_{2A}$. Double labeling experiments of sst_{2A} and TH showed no colocalization in either species. In the mouse, sst_2 immunoreactivity was localized to rod bipolar, horizontal and TH- and glycine-containing amacrine cells (Cristiani et al., 2002), employing the sheep polyclonal antibody mentioned above (Schindler et al., 1997).

In human ocular tissue, sst_2 immunostain was reported in the outer and inner segments of rods and cones and individual cells in the ONL, INL and GCL (Klisovic et al., 2001). The morphology of the individual cells (amacrine, rod bipolar cells, etc.) in the retinal layers mentioned was not assessed. sst_2 immunostain was also detected in endothelial cells of blood vessels, as well as on cell membranes and cytoplasm of individual RPE cells in culture. A rabbit polyclonal antibody raised against the N-terminal 45 amino acids of the sst_2 was employed. This amino acid sequence is common to both sst_{2A} and sst_{2B}, and thus one could suggest the presence of both splice variants of the sst_2 receptor. Also, in human retina sst_{2A} immunoreactivity was reported in the inner and outer nuclear and plexiform layers, as well as the RPE (Van Hagen et al., 2000). These findings were confirmed by RT-PCR studies (Klisovic et al., 2001; Van Hagen et al., 2000), as mentioned previously.

In the lower vertebrates, sst_{2A} immunoreactivity was localized to both inner and outer retina of the salamander (Akopian et al., 2000), using the same antibody employed by Johnson et al. (1998, 1999). Specifically, sst_{2A} immunoreactivity was observed in rod and cone photoreceptors with prominent staining through out the inner segment and synaptic terminals. sst_{2A} immunoreactivity was also noted in bipolar and amacrine cell

bodies, in the OPL and in all laminae of the IPL. Diffuse immunostain was reported in the GCL. In retinas of the adult newt Pl. waltl, sst_{2A} immunoreactivity was also observed in rod bipolar, inner segments of the cone photoreceptors and in the region corresponding to connecting cilia of rods (Grigoryan, Vasilaki, Mastrodimou, & Thermos, 2003).

2.3. sst_{2B}

There are not many reports supporting the presence of sst_{2B} in the retina. sst_{2B} immunoreactivity was localized to rod and cone photoreceptors of the rat, but not the rabbit retina (Vasilaki et al., 2001). A rabbit polyclonal antibody raised against the peptide fragment of the carboxy terminus [amino acids 329–343] of the $rsst_{2B}$ was employed (Schulz et al., 2000). Other investigators have reported the absence of the sst_{2B} receptor subtype. Petrucci et al. (2001) could not detect sst_{2B} immunoreactivity in the rabbit retina using a rabbit antiserum raised against the 15 terminal amino acids of the carboxy terminus of the rat sst_{2B} plus an extra cysteine residue at the amino terminal end (Schindler, Humphrey, Lohre, & Friauf, 1999), while Johnson et al. (1999) could not detect sst_{2B} mRNA in the retina of the rat. More recent findings have shown the presence of sst_{2B} in individual human RPE cell cultures (Papadaki, Vasilaki, Tsilimbaris, Pallikaris, & Thermos, 2002). As indicated above, Klisovic et al. (2001) reported sst_2 immunoreactivity in RPE cultures that may be partly due to sst_{2B}.

2.4. sst_3

sst_3 immunoreactivity has not been localized in mammalian retina, even though its mRNA was detected by RT-PCR in rat (Mori et al., 1997) and human retina (Klisovic et al., 2001; Van Hagen et al., 2000). sst_3 immunostain was reported in the lower vertebrate newt (*Pleurodeles waltlii* Michan) to be most intensely present in the inner segments of cones and in cilia of rods (Grigoryan et al., 2003).

2.5. sst_4

We recently examined sst_4 immunoreactivity in the rat retina. It was confined to multistratified processes of the IPL, the plasma membrane, the cytoplasm of cell bodies and clusters of long processes in the GCL. Optic nerve fibers of the rat retina were also selectively immunostained for sst_4. Colocalization of sst_4 and the ganglion cell marker MAP-1A was observed in both cell bodies and processes of the GCL and processes in the IPL. This colocalization signifies that the sst_4 expression is restricted in ganglion cell bodies, the dendritic field and axons, and not on amacrine cell processes in the IPL or the cell bodies of displaced amacrine cells in the GCL (Vasilaki et al., 2002).

Brecha, Vila, and Allen (2002) in a recent report presented evidence of specific sst_4 immunoreactivity in the mouse and rat retina to be localized to multistratified ganglion cells and in processes in all regions of the IPL. Also, sst_4 immunoreactivity was localized to calbinding (CaBP)-IR and CaBP-non-IR cells in the GCL of the mouse retina (Cristiani et al., 2002). RT-PCR data also support the presence of sst_4 in rat (Mori et al., 1997), mouse (Cristiani et al., 2002), and human retina (Klisovic et al., 2001).

2.6. sst_5

sst_5 immunoreactivity has not been reported in the retina of any species even though low sst_5 mRNA levels were detected by RT-PCR in rat (Mori et al., 1997), mouse (Cristiani et al., 2002), and human retina (Klisovic et al., 2001). We have data to support sst_5 immunoreactivity in human RPE cell cultures (Vasilaki et al., unpublished data).

2.7. Summary

An attempt was made to present the most updated evidence on the presence and localization of somatostatin receptors in the retina. The antibodies employed by the different investigators and their findings regarding the localization of each receptor subtype were compared (Fig. 1, Table 1). An overall general consensus on the localization of the receptors is observed in the different species studied.

Due to the earlier availability of the sst_2/sst_{2A} antibodies a larger number of investigations focused on these receptors. Most of these studies support the presence of sst_{2A} in rod bipolar cells in all species studied (Akopian et al., 2000; Cristiani et al., 2002; Fontanesi et al., 2000; Johnson et al., 1996, 1998; Johnson, Rickman, & Brecha, 2000; Petrucci et al., 2001; Vasilaki et al., 2001), with the exception of the study by Helboe and Moller (1999). Minor discrepancies are apparent in results obtained within the same group and between groups. Thus, Johnson et al. (1999) reported the presence of sst_{2A} in cone photoreceptors, cone bipolar and horizontal cells of the rat but not the rabbit retina (Johnson et al., 1998), employing the same conditions. Also, Vasilaki et al. (2001) reported the presence of sst_{2B} in rat, but not rabbit, rod and cone photoreceptors. These discrepancies allude to species differences.

The major discrepancies between groups pertain to the presence (Cristiani et al., 2000) or absence (Helboe & Moller, 1999; Vasilaki et al., 2002) of sst_1 immunoreactivity in TH-containing amacrine cells and the presence (Cristiani et al., 2002; Fontanesi et al., 2000; Helboe & Moller, 1999; Johnson et al., 1999) or absence

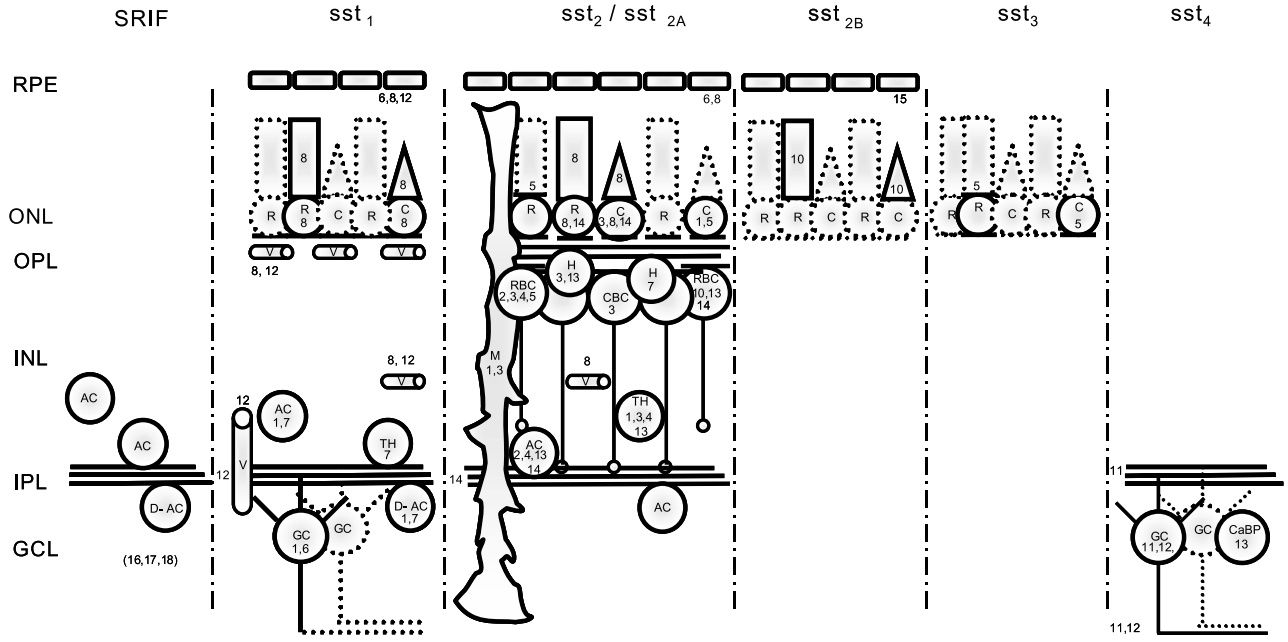

Fig. 1. Mapping of ssts in the retina. This figure summarizes the results of different investigations on the localization of somatostatin receptor subtypes in retinal circuitry and the RPE. A panel is also included to depict the localization of somatostatin neurons. RPE, retinal pigment epithelium, ONL, outer nuclear layer, OPL, outer plexiform layer, INL, inner nuclear layer, IPL, inner plexiform layer, GCL, ganglion cell layer, R, rod, RBC, rod bipolar cell, C, cone, CBC, cone bipolar cell, H, horizontal cell, V, vessel, AC, non-TH amacrine cell, TH, tyrosine hydroxylase containing amacrine cell, D-AC, displaced amacrine cell, GC, ganglion cell, Ca-BP, calbindin expressing ganglion cell, M, Mueller cell. (1) Helboe and Moller (1999), (2) Johnson et al. (1998), (3) Johnson et al. (1999), (4) Fontanesi et al. (2000), (5) Grigoryan et al. (2003), (6) Van Hagen et al. (2000), (7) Cristiani et al. (2000), (8) Klisovic et al. (2001), (9) Petrucci et al. (2001), (10) Vasilaki et al. (2001), (11) Brecha et al. (2002), (12) Vasilaki et al. (2002), (13) Cristiani et al. (2002), (14) Akopian et al. (2000), (15) Papadaki et al. (2002), (16) Sagar and Marshall (1988), (17) White et al. (1990), (18) Larsen et al. (1990).

Table 1
Antibodies used for the locallization of sst_s in the retina

sst	Antigen	Reference
sst_1	C-terminus 374–391 (human)	Cristiani et al. (2000), Helboe and Moller (1999), Helboe et al. (1997)
	C-terminus 374–391 (rat)	Schulz et al. (2000), Vasilaki et al. (2002)
	N-terminus 1–57 (human)	Albers et al. (2000), Klisovic et al. (2001)
sst_2	N-terminus 1–45 (human)	Albers et al. (2000), Klisovic et al. (2001)
sst_{2A}	C-terminus 361–369 (mouse)	Akopian et al. (2000), Johnson et al. (1998, 1999)
	C-terminus 347–366 (mouse, rat, human)	Fontanesi et al. (2000), Petrucci et al. (2001), Schindler et al. (1997)
	C-terminus 330–369 (human)	Helboe et al. (1997)
	C-terminus 355–369 (rat)	Schulz et al. (2000), Vasilaki et al. (2001)
sst_{2B}	C-terminus 329–343 (rat)	Schulz et al. (2000), Vasilaki et al. (2001)
	C-terminus 329–343 +cys (rat)	Petrucci et al. (2001), Schindler et al. (1999)
sst_3	C-terminus 417–428 (rat)	Grigoryan et al. (2003)
sst_4	C-terminus 362–384 (mouse)	Cristiani et al. (2002), Schulz et al. (2000), Vasilaki et al. (2002)

(Johnson et al., 1998; Vasilaki et al., 2001) of colocalization of sst_{2A} and TH. While some of the discrepancies may be due to the different antibodies employed, one must take into account that differences were also observed among investigators employing the exact same antibody in the same species (Cristiani et al., 2000; Helboe & Moller, 1999). It is the opinion of the author that these discrepancies are due to minor technical differences that are difficult to pinpoint. The major issue involving the function of these receptors will be elucidated with direct pharmacological studies examining the effect of sst_1 and sst_2 selective agonists on the release of dopamine.

3. Functional role of somatostatin receptors in retinal circuitry

The presence of somatostatin receptors in photoreceptors, rod bipolar and ganglion cells suggests that somatostatin synthesized and released by a subcategory of amacrine cells may act at a distance in a paracrine fashion, and may influence the release of glutamate the major neurotransmitter of these retinal cell types. More specifically, Akopian et al. (2000) reported the somatostatin modulation of voltage-gated K^+ and Ca^{2+} currents through activation of sst_{2A} receptors present in the photoreceptors of the salamander retina, and suggested a possible role for somatostatin in the regulation of glutamate transmitter release from photoreceptors. Also, studies performed on isolated rod bipolar cells of the rabbit retina showed SRIF and octreotide to modulate Ca^{2+}-voltage-dependent K^+ channels by activating sst_2 receptors (Petrucci et al., 2001). Somatostatin was also shown to inhibit calcium influx into rat bipolar cell axonal terminals (Johnson, Caravelli, & Brecha, 2001). The sst_2 receptor couples to $G_{0\alpha}$, localized in the IPL in the rabbit retina (Vasilaki, Georgoussi, & Thermos, 2003). This relationship may be instrumental in somatostatin's modulation of the ion channels found on rod bipolar cells and the subsequent neurotransmitter release. Earlier electrophysiological studies have shown that $G_{0\alpha 2}$ couples SRIF receptors to Ca^{2+} channels (Kleuss et al., 1992). No studies have been reported to date, however, to substantiate a direct regulation of glutamate release by somatostatin in the retina.

The presence of sst_1 and sst_2 on somatostatin and TH expressing amacrine cells in the rat retina, respectively, suggests a role for somatostatin in the regulation of its own release as well as that of dopamine. To directly study the former, namely whether sst_1 functions as an autoreceptor in the retina, we have conducted functional studies ex vivo in retinal explants to measure the effect of the sst_1 selective agonist CH275 on somatostatin release. We have preliminary data depicting a decrease of SRIF release upon CH275 activation, suggesting an autoreceptor function for sst_1 (N. Mastrodimou, unpublished data).

As presented above, different groups have shown the presence (Helboe & Moller, 1999; Johnson et al., 1998, 1999) or absence (Johnson et al., 1998; Vasilaki et al., 2001) of sst_2 and sst_{2A} colocalization with TH, yet no functional data have been presented to date to support a direct regulation of dopamine release by somatostatin in the retina.

We recently focused our research interests to the putative somatostatin–nitric oxide interactions for the following reasons. In brain, SRIF is colocalized with NADPH-diaphorase (a marker for nitric oxide synthase, Dawson, Bredt, Fotuhi, Hwang, & Snyder, 1991) in medium sized aspiny neurons of the striatum (Vincent & Johansson, 1983), but there are no reports to suggest that the two systems interact functionally. NADPH-diaphorase is present in all retinal cell types, including subpopulations of amacrine cells. While colocalization of SRIF with NO in amacrine cells was never observed (Koistinaho & Sagar, 1995), as was the case in the striatum (Vincent & Johansson, 1983), the sst_{2A} receptor appears to be localized in most retinal cells (Akopian et al., 2000; Helboe & Moller, 1999; Johnson et al., 1998, 1999) that are known to express nitric oxide synthase (NOS) or NADPH-diaphorase (Haverkamp & Eldred, 1988; Koch, Lambrecht, Haberecht, Redburn, & Schmidt, 1994; Osborne, Barnett, & Herrera, 1993; Perez, Larsson, Alm, Andersson, & Ehinger, 1995; Vasilaki et al., 2001). Thus, it appeared of particular interest to examine whether SRIF acting initially via sst_{2A} and subsequently via the other receptor subtypes could influence nitric oxide production. We investigated the putative coexistence of the somatostatin receptor subtypes sst_1–sst_5 with NADPH-diaphorase, and their ability to influence nitric oxide levels in the retina.

sst_{2A} was colocalized with NADPH-diaphorase on rod bipolar cells of the rabbit retina, while sst_{2B} is colocalized with NADPH-diaphorase on the photoreceptors of the rat. These results supported for the first time a possible role of SRIF in the regulation of nitric oxide in the retina (Vasilaki et al., 2001). The colocalization of NADPH-diaphorase with the other SRIF receptor subtypes was subsequently examined. sst_1 immunofluorescence was colocalized with NADPH-diaphorase mainly in the RPE, and blood vessels of the inner retina while sst_4 immunoreactivity was colocalized with NADPH-diaphorase in ganglion cell bodies (Vasilaki et al., 2002).

There are few studies measuring nitric oxide levels in the retina. Neal, Cunningham, and Matthews (1998) demonstrated that nitric oxide was selectively released from retinal amacrine and bipolar cells in the rabbit retina, depending on the light stimulus employed. We conducted ex vivo experiments in retinal explants (RPE excluded), in order to investigate directly SRIF's regulation of nitric oxide production. The levels of NO in rat retinal explants were assessed by the production of its stable metabolites NO_2^- and NO_3^- (NO_x^-) by the method of Grisham, Johnson, and Lancaster (1996). Somatostatin increased NO_x^- basal levels in a concentration-dependent manner. To assess which somatostatin receptor subtype was responsible for this effect, selective analogs for all the receptor subtypes were employed. In the neural retina of the rat, sst_{2B} (Vasilaki et al., 2001) and sst_4 (Vasilaki et al., 2002) are colocalized with NADPH-diaphorase, and their activation would be expected to influence nitric oxide synthase. However, only the sst_2 specific analog (L-779,976; Rohrer et al., 1998) was able to increase NO_x^- levels. The sst_2 antagonist CYN-154806 (Feniuk, Jarvie, Luo, & Humphrey, 2000),

was able to block the L-779,976 effect in retinal explants, supporting the pharmacological relevance of the sst_2 effect (Vasilaki et al., 2002).

We extended the above studies to the RPE, a monolayer of cells that is crucial for the integrity of the retina. As mentioned in the previous section, Klisovic et al. (2001) detected the sst_1 and sst_2 genes and immunoreactivity in cultured RPE cells. We have data to support the presence of SRIF and its receptor subtypes sst_{2B} and sst_5 in human RPE cell cultures. Subsequent studies also showed the presence of NADPH-diaphorase. As in the retinal explants SRIF, the sst_2 selective analogue MK678 increased the levels of NO_x^- in a concentration-dependent manner (Papadaki et al., 2002).

Nitric oxide is believed to play a variety of roles in ocular physiology. These include the maintenance of tight junction integrity (Zech et al., 1998), the development of immune and inflammatory responses and blood flow (Cai & Boulton, 2002; Dezsi, Dornyei, Szentivanyi, Tulassay, & Monos, 1997; Holtkamp, Kijlstra, Peek, & de Vos, 2001). Our data suggest that somatostatin may influence the above functions by its regulation of nitric oxide production.

4. Pharmacology of somatostatin in the retina and putative therapeutic in retinal disease

Somatostatin has been studied as a possible therapeutic agent in the treatment of major retinal diseases such as proliferative diabetic retinopathy (PDR), age-related macular degeneration (ARMD) and cystoid macular oedema (CME), diseases whose underlying complications involve retinal neovascularization and oedema that lead to blindness.

In the early nineties somatostatin and analogues were employed in clinical studies for the treatment of PDR, a disease whose major clinical treatment even today involves laser ablation of the retinal vasculature. Somatostatin's therapeutic use was based on the ability of somatostatin to inhibit growth hormone secretion, a hormone implicated in the pathogenesis of diabetic retinopathy (Kirkegaard, Norgaard, Snorgaard, Larsen, & Lund-Andersen, 1990; Mallet et al., 1992; McCombe, Lightman, Eckland, Hamilton, & Lightman, 1991).

Ocular neovascularization is strongly associated with retinal ischemia the underlying cause of diabetic retinopathy (Cohen, Jasti, & Rye, 1977). Growth hormone and the peptide growth factors such as IGF-1, fibroblast growth factor (bFGF), platelet-derived growth factor (PDFG), and vascular derived growth factor (VEGF) have been implicated in the development of retinal neovascularization and the pathogenesis of ischemia (Cai & Boulton, 2002; Dills, Moss, Klein, & Klein, 1991; Grant, Caballero, & Millard, 1993; Hyer, Sharp, Brooks, Burrin, & Kohner, 1989; Kirkegaard et al., 1990; Merimee, Zapf, & Froesch, 1983; Paques, Massin, & Gaudric, 1997).

To investigate the role of the GH-IGF-somatostatin axis in ischemia-induced retinal neovascularization (mouse model of oxygen induced retinopathy) and its interactions with VEGF, Smith et al. (1997) employed mice with experimentally altered levels of GH and in normal mice that were given the sst_2 selective agonist MK678. The conclusion was that inhibitors of GH and IGF-1 might be useful for the inhibition of neovascularization. Employing the same model of oxygen induced retinopathy, Higgins, Yan, and Schrier (2002) showed that octreotide and a new somatostatin agonist Woc4D inhibited retinal neovascularization. In addition, Klisovic, Sall, O'Dorisio, Katz, and William (2001) reported that somatostatin and octreotide inhibited the IGF-1 mediated tyrosine autophosphorylation of the IGF-receptor and VEGF gene transcription in RPE cells through an sst_2 mechanism. The investigators suggested the usefulness of these agents in the treatment of PDR.

Recent clinical investigations also focus on the use of somatostatin receptor agonists as therapeutic agents for diabetic retinopathy (Davis, Wilson, & Grant, 2001; Grant et al., 2000; Simo et al., 2002). Grant et al. (2000) performed a randomised controlled clinical study and examined the efficacy of octreotide (sst_2 agonist) in the therapy of patients with severe non-proliferative and early proliferative diabetic retinopathy. The investigators suggest that octreotide treatment in euthyroid patients may retard progression of advanced diabetic retinopathy and may delay the time of laser surgery. Octreotide also reduced the number of vitreous haemorrhages after full scatter laser coagulation in a randomised trial in diabetic patients with high-risk proliferative retinopathy (Boehm, Lang, Jehle, Feldman, & Lang, 2001), while Spranger et al. (2001) suggest the use of combined treatment (somatostatin analogues and retinal photocoagulation) of PDR.

ARMD is the major causes of blindness in people over 50 years of age. The pathogenesis of the disease is not understood yet newly formed vessels from the underlying choroids grow beneath the RPE, and angiogenesis in choroidal neovascularization is morphologically described secondary to ARMD (D'Amore, 1994). Two types of the disease have been reported, the atrophic form and the neovascular. The neovascular form is characterized by leakage of blood or serum, the detachment of the RPE and choroidal neovascularization, which lead to scarring of the macula. The atrophic form is not characterized by leakage of blood but it involves choriocapillaries, RPE and the photoreceptors. Both forms of the disease can lead to loss of vision. The RPE is involved in the pathogenesis of ARMD and there are reports to suggest that octreotide use in choroidal neovascular membranes may be successful not only because it may inhibit angiogenesis, but also due to

its antiproliferative actions (Lopez et al., 2001), which result in inhibition of RPE proliferation and migration (Spraul, Kaven, Kampmeier, Lang, & Lang, 1999).

In a recent review on somatostatin and its receptors in retinal disease, Van Hagen et al. (2000) reported the successful treatment of 13 eyes with neovascular ARMD with the somatostatin analogue octreotide (Sandostatin-Lar). In a pilot clinical study, Papadaki et al. (2002) examined the role of lanreotide in the treatment of choroidal neovascularization secondary to ARMD and reported a trend towards stabilization of the disease. sst_{2A} receptors were found in newly formed endothelial and fibroblast-like cells in fibrovascular choroidal neovascular membranes (Lambooij et al., 2000). The presence of somatostatin and its receptors (sst_{2B}) was confirmed in human RPE cells (Papadaki et al., 2002), while the sst_1 receptor was localized in rat RPE (Vasilaki et al., 2002). These reports provide promising results for the use of sst_2 selective agonists, such as octreotide and lanreotide, in the treatment of neovascular ARMD, while the therapeutic use of sst_1 agonists should also be explored.

The RPE is also involved in cystoid macular disease, since a dysfunctional RPE disrupts the outer blood—retinal barrier leading to the movement of small molecules, ions and fluids between the retina and the choroid and macular oedema. CME has been treated successfully with octreotide as reported by Kuijpers, Baarsma, and van Hagen (1998). The integrity of both the outer and inner blood—retinal barrier is affected in CME. The disruption of the capillary endothelium and the subsequent leakage from the capillaries surrounding the macula destroys the inner blood—retinal barrier. This may occur in response to ischemia, but also to inflammation and other conditions. Somatostatin receptors are differentially expressed in human vascular tissue (Curtis et al., 2000). Studies conducted in rapidly proliferating human retinal endothelial cells that were stimulated by IGF-I and bFGF in vitro reported a direct antiproliferative effect of octreotide (Grant et al., 1993).

The effect of octreotide on the growth of RPE cells in culture has also been examined in order to assess the usefulness of somatostatin agents in proliferative vitreoretinopathy (Luo, Peyman, Conway, & Woltering, 1996).

5. Conclusions

The aim of this review was to summarize the knowledge acquired to date on somatostatin and its role in the retina. The experimental data presented and discussed provide solid evidence that somatostatin may act in an autocrine or paracrine manner to influence RPE and retinal physiology. The differential localization of its receptor subtypes in the RPE, photoreceptors, rod bipolar cells, blood vessels of the inner retina (Fig. 1) suggest a role for somatostatin in proper RPE physiology, the well being of the blood-eye barrier, retinal blood flow, and the regulation of neurotransmitter release. The correlation of somatostatin's actions in the retina with specific receptor subtypes still remains a target of investigation, while SRIF's ability to regulate nitric oxide production supports the strategic planning of studies to delineate the SRIF-NO relationship and its importance in physiological and diseased retina.

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