CHAPTER 8

SOMATOSTATIN RECEPTOR 2A EXPRESSION IN CHOROIDAL NEOVASCULARIZATION SECONDARY TO AMD

ABSTRACT

Purpose: The growth of ocular neovascularization is regulated by a balance between stimulating and inhibiting growth factors. Somatostatin effects angiogenesis by inhibiting the growth hormone/insulin-like growth factor axis and also has a direct anti-proliferative effect on human retinal endothelial cells. The purpose of our study is to investigate the expression of somatostatin receptor (sst) subtypes and particularly sst subtype 2A (sst_{2A}) in normal human macula, and to study sst_{2A} in different stages of age-related maculopathy (ARM), because of the potential anti-angiogenic effect of somatostatin analogues.

Methods: Sixteen eyes (10 enucleated eyes, 4 donor eyes and 2 surgically removed choroidal neovascular (CNV) membranes) of 15 patients with eyes at different stages of ARM were used for immunohistochemistry. Formaldehyde-fixed paraffin-embedded slides were incubated with a polyclonal anti-human sst_{2A} antibody. mRNA expression of five sst subtypes and somatostatin was determined in the posterior pole of 3 normal human eyes by reverse transcriptase-polymerase chain reaction.

Results*:* The immunohistochemical expression of sst_{2A} in newly formed endothelial cells and fibroblasts-like cells was strong in fibrovascular CNV membranes. mRNA of sst subtypes 1, 2A and 3, as well as somatostatin, was present in the normal posterior pole; sst subtypes 4 and 5 were not detectable.

Conclusions*:* Most early-formed CNV in ARM express sst_{2A}. We confirmed the presence of mRNA of sst subtype 2A in normal human macula, and demonstrated that also subtype 1 and 3, as well as somatostatin, are present. Sst_{2A} receptors bind potential anti-angiogenic somatostatin analogues such as octreotide. Therefore, somatostatin analogues may be an effective therapy in early stages of neovascular AMD.

INTRODUCTION

Age-related maculopathy (ARM) is the major cause of blindness in people over 65 years of age in the Western world. The prevalence of ARM is up to 14% in people more than 85 years.[3] Late stages of ARM, also called age-related macular degeneration (AMD), include geographic atrophy and neovascular macular degeneration. The neovascular form is characterized by choroidal neovascularization (CNV) and is responsible for 80% of cases of severe vision loss.[3] These numbers will increase because of the increasing age of the population. In CNV, newly formed vessels from the underlying choroid grow beneath the retinal pigment epithelium (RPE) and the retina.[251] Although the morphology of angiogenesis in CNV secondary to AMD has been described in detail, the pathogenesis is still poorly understood. A balance between a number of stimulating and inhibiting growth factors regulates the growth of neovascularization.[251] Vascular endothelial growth factor (VEGF), an endothelial specific mitogen, is regarded as one of the most important ocular angiogenic factors, especially in ischemic disease.[144,145,148,159,251,290,291] Other regulating growth factors include fibroblast growth factors (FGFs), transforming growth factor-β (TGF-β) and insulin-like growth factor-I (IGF-I). Most of these growth factors are shown to be upregulated in a diversity of cells (RPE, fibroblasts, capillary endothelial cells) involved in CNV.[145,159,160,189,190,246,247]

Recently, it has been shown in a transgenic mouse model that inhibition of growth hormone (GH), mediated by IGF-I, can inhibit ischemia-induced retinal neovascularization in vivo.[14] GH secretion is inhibited by somatostatin and somatostatin analogues. Systemic treatment with a somatostatin analogue diminished the level of ocular neovascularization in mice.[177]

Somatostatin binds with high affinity to 5 subtype receptors (sst_1 to sst_5). These receptors were identified in various animal retinas.[292-294] The exact role of a direct receptor-mediated effect by somatostatin analogues is still unknown. To date, information about sst_2 receptor expression in CNV is not available, and until now sst subtype expression has not been described in normal human retina.

The purpose of our study was to investigate the expression of somatostatin receptor 2A (sst_{2A}) in different stages of ARM, and the expression of sst subtypes and somatostatin in normal human macula.

MATERIALS AND METHODS

The study was performed according to the tenets of the Declaration of Helsinki. Enucleation or surgical excision of subfoveal CNVs was performed after obtaining informed consent of the patient.

Patients

All eyes were retrieved from the files from the Ophthalmic Pathology Department of the University Hospital of Rotterdam. Sixteen eyes (10 enucleated eyes, 4 donor eyes and 2 surgically removed subretinal neovascular membranes) of 15 patients with eyes at different stages of ARM were used for immunohistochemistry. The description of each eye is given in Table 8.1. Eight eyes (of 7 patients) had clinical diagnoses of AMD. In 8 other eyes, ARM was diagnosed histopathologically according to the following criteria: Early stages of ARM (n=3) were characterized by the presence of basal laminar deposits, basal linear deposits (BLD), soft drusen, and thickening of Bruch's membrane.[112] Neovascular AMD (n=12) was classified as sub-RPE CNV, subretinal CNV (between neuroretina and RPE) or mixed sub-RPE and subretinal CNV.[120,295] Photoreceptors, Bruch's membrane and BLD were helpful in the orientation of the specimens.[120] Sub-RPE CNV and mixed CNV, or subretinal CNV in elderly patients in the presence of BLD or soft drusen were classified as CNV secondary to AMD.[120] In CNV, we recorded the presence of fibrovascular or fibrocellular tissue, hemorrhage, vascular endothelium, BLD and RPE.[120] One eye was classified as non-neovascular (geographic) AMD. Eight enucleated eyes without ARM (donor eyes or enucleated for other reasons) were used as controls (Table 8.2). The eyes were processed for routine diagnostic procedures by fixation in formaldehyde and were embedded in paraffin.

Immunohistochemistry

Rabbit anti-human sst_{2A} polyclonal antibody (R2-88) was kindly provided by Dr. A. Schonbrunn (Department of Integrative Biology and Pharmacology, University of Texas Houston Medical School, USA). The antibody was raised against a 22-amino acid peptide located at the C-terminal region of the sst_2 receptor. The sst_{2A} antibody had been characterized and tested before by Western blot analysis and peptide binding.[296,297] Mouse monoclonal antibody against smooth muscle actin (SMA) was obtained from Biogenex (San Ramon, CA, USA) and mouse monoclonal antibody against macrophages (CD68) from Dako (Glastrup, Denmark). Five μm sections were prepared. The sections were deparaffinated, rehydrated and (for sst_{2A} and CD68)

TABLE 8.1 PATIENT MATERIAL AND SST_{2A} RECEPTOR EXPRESSION IN EYES WITH ARM

No.	Age/ sex	OD/ OS	Clinical description	Histological classification	Sst_{2A} expression*						
					Preexistent tissue			Neovascular tissue			
								FV		FC	
					RPE	CC	CH	EC#	FBL	EC#	FBL
ARM1	85/M	OS	necrotising sclerokeratomalacy	early ARM: BLD	++	0	++	.	.	.	.
ARM2	98/F	OS	corneal ulcer	early ARM: confluent soft drusen	++	+	++	.	.	.	.
ARM3	96/F	OD	staphyloma, suspected ciliary body melanoma	early ARM: BLD; glaucoma; corneal ulcer	++	0	+	.	.	.	.
ARM4	77/M	OS	neovascular glaucoma	nonneovascular AMD; early geographic atrophy; occlusion central retinal artery; ischemic retinal disease	++	0	+	.	.	.	.
CNV1	79/M	U	surgically excised CNV	mixed CNV, FV and FC, hemorrhage	NP	NP	NP	37/48	++	NP	0
CNV2	79/F	U	surgically excised CNV	subretinal CNV, FV and FC, hemorrhage	NP	NP	NP	15/18	++	NP	++
CNV6	72/M	OS	disciform MD	mixed CNV, BLD, FV and FC, hemorrhage	+	0	+	28/50	+	0/7	0
CNV7	86/M	OS	disciform MD, acute glaucoma	sub-RPE CNV, BLD, FV and FC, hemorrhage; retinal detachment; posterior uveitis	++	+	++	NP	NP	2/4	++
CNV8	91/M	OS	donor eye	disciform MD, mixed CNV, BLD, FC	NC	0	NC	.	.	0/6	+
CNV9	87/M	OS	donor eye	disciform MD, mixed CNV, BLD, FV and FC	++	+	++	11/16	+	3/5	+
CNV10	83/M	OD	painful eye, suspected uveal melanoma	ischemic retinal disease; disciform MD, mixed CNV, BLD, FV and FC, hemorrhage	++	0	+	26/64	++	0/3	++
CNV11	73/M	OS	disciform MD	subretinal CNV, FC and FV	++	0	+	13/15	++	NC	+
CNV12	73/M	OD	disciform MD, post irradiation	subretinal CNV, FV	+	0	++	2/3	+	.	.
CNV13	82/M	OD	disciform MD	mixed CNV, confluent soft drusen, FC	+	0	0	.	.	13/36	0
CNV14	85/F	OS	post surgical endophthalmitis	subretinal CNV, FV, endophthalmitis, uveitis	+	0	+	2/2	+	.	.
CNV17	84/F	OS	disciform MD	mixed CNV, FV and FC, BLD, hemorrhage	+	0	+	0/2	+	NC	0

**Categories of sst_{2A} expression: 0 = 0 – 10% positive cells; + = 11 – 50% positive cells; ++ = 51 – 100% positive cells. #Sst_{2A} expression in endothelial cells in CNV was quantitatively determined by counting the proportion of positive vessels in randomly selected sections. (MD = macular degeneration; mixed CNV = mixed subretinal and sub-RPE CNV; FV = fibrovascular CNV; FC = fibrocellular scar; BLD = basal laminar deposits; RPE = retinal pigment epithelium; CC = choriocapillaris; CH = choroidal vessels; CNV = choroidal neovascularization; EC = endothelial cells; FBL = fibroblasts-like cells. U = unknown; NC = not classifiable; NP = not present)*

TABLE 8.2. PATIENT DATA AND SST RECEPTOR SUBTYPE EXPRESSION IN NORMAL EYES

No	Age/ sex	OD/ OS	Clinical description	Sst receptor subtype expression* (RT-PCR)							Sst_{2A} expression† (Immunohistochemistry)		
				Sst_1	Sst_{2A}	Sst_3	Sst_4	Sst_5	SS14	HPRT	RPE	CC	CH
1	71/U	OD	donor eye	.	.	.	.	.	.	.	++	+	++
2	51/M	OD	ciliary body melanoma	.	.	.	.	.	.	.	+	0	+
3	78/M	OS	choroidal melanoma	.	.	.	.	.	.	.	++	0	+
4	81/M	OS	tarsal squamous cell carcinoma	.	.	.	.	.	.	.	+	+	++
5	42/M	OS	choroidal melanoma	.	.	.	.	.	.	.	++	0	++
6	76/F	OS	choroidal melanoma	.	.	.	.	.	.	.	++	0	++
7	57/M	OS	recurrent conjunctival melanoma	.	.	.	.	.	.	.	+	0	+
8	60/M	OS	choroidal melanoma	.	.	.	.	.	.	.	++	0	++
9	69/M	OD	ciliary body adenoma	+	+	+	-	-	+	+	.	.	.
10	78/M	OS	spindle cell nevus	+	+	+	-	-	+	+	.	.	.
11	26/M	OS	choroidal melanoma	+	+	+	-	-	+	+	.	.	.

**Categories of sst subtype expression (RT-PCR): - = no expression, + = positive expression. †Categories of sst_{2A} expression (immunohistochemistry): 0 = 0 – 10% positive cells; + = 11 – 50% positive cells; ++ = 51 – 100% positive cells. (SS14 = somatostatin; HPRT = hypoxanthine-guanine phosphoribosyl transferase; RPE = retinal pigment epithelium; CC = choriocapillaris; CH = choroidal vessels. U = unknown)*

microwave heated for 10 minutes. After the slides were blocked with normal goat serum (Dako, 1:10) for 15 minutes, they were incubated with the sst_{2A} antibody (1:1000) or CD68 antibody (1:2000) overnight at 4°C, or with anti-SMA (1:150) for 1 hour at room temperature. The sections were further incubated with biotinylated multilink antibodies for 30 minutes, followed by alkaline phosphatase-labeled antibiotin (both from Biogenex) for 30 minutes. The bound antibodies were visualized by incubating the sections with new fuchsin for 30 minutes in the dark. The slides were counterstained with Mayer's hematoxylin, mounted and examined by light microscopy. We determined the sst_{2A} expression quantitatively in endothelial cells of CNV by counting the proportion of positive vessels in randomly selected sections. The total number of counted vessels was pooled, and the proportions of positive cells in fibrovascular and fibrocellular CNV were compared with χ^2 analysis. For other tissue components, we semi-quantitatively graded sst_{2A} expression in 3 categories: 0 (0 – 10% positive cells), 1 (11 – 50% positive cells) and 2 (51 – 100% positive cells). Negative controls for immunohistochemistry included 1) omission of the primary antibody, 2) use of an irrelevant antibody of the same isotype, and 3) preabsorbtion of the sst_{2A} antibodies with the immunizing receptor peptide for 4 hours at a concentration of 3 µg/ml.

RT-PCR

In order to study the mRNA expression of sst subtypes in normal human eyes, posterior poles from three eyes (Table 8.2) were dissected directly after enucleation. A sample of about 0.2 mm^2 located in the macula, including RPE, choroid and sclera, was snap frozen in liquid nitrogen. RT-PCR was performed as described before[298] but with different primers (Table 8.3).

Several controls were included in the RT-PCR experiments. To ascertain that no detectable genomic DNA was present in the $polyA^+$ mRNA preparation (because the sst genes are intronless), the cDNA reactions were also performed without reverse transcriptase and amplified with each primer pair. Amplification of the cDNA samples with the hypoxanthine-guanine phosphoribosyl transferase (HPRT) specific primers served as positive control for the quality of the cDNA. To exclude contamination of the PCR reaction mixtures, the reactions were also performed in the absence of DNA template in parallel with cDNA samples. As a positive control for the PCR reactions of the sst receptor subtypes, 0.1 to 0.001 µg of human genomic DNA, representing approximately 30.000 to 300 copies of sst-template, was amplified in parallel with the cDNA samples. As a positive control for the PCR of HPRT and somatostatin cDNA, aliquots of a cDNA sample known to contain somatostatin and HPRT mRNA were amplified, because these primer pairs did enclose introns in the genomic DNA.

TABLE 8.3 PRIMERS USED FOR RT-PCR ANALYSIS

receptor	primer	sequence (5' –3')*	product size (base pair)
sst_1	forward	ATGGTGGCCCTCAAGGCCGG	318
	reverse	CGCGGTGGCGTAATAGTCAA	
sst_{2A}	forward	GCCAAGATGAAGACCATCAC	414
	reverse	GATGAACCCTGTGTACCAAGC	
sst_3	forward	CCAACGTCTACATCCTCAACC	314
	reverse	TCCCGAGAAGACCACCAC	
sst_4	forward	ATCTTCGCAGACACCAGACC	321
	reverse	ATCAAGGCTGGTCACGACGA	
sst_5	forward	CGTCTTCATCATCTACACGG	226
	reverse	CCGTCTTCATCATCTACACGG	
SS14	forward	GATGCTGTCCTGCCGCCTCCAG	349
	reverse	ACAGGATGTGAAAGTCTTCCA	
HPRT	forward	CAGGACTGAACGTCTTGCTC	413
	reverse	CAAATCCAACAAAGTCTGGC	

The sequences of the primers for sst_1 were derived and adapted from Wulfsen et al.,[41] for sst_5 from Kubota et al.,[42] and all others were designed by use of the Primer3! software (http://www.genome.wi.mit.edu/genome_software/other/primer3.html) and the appropriate GenBank entries. (SS14 = somatostatin; HPRT = hypoxanthine-guanine phosphoribosyl transferase)

RESULTS

Immunohistochemistry

In normal retina (n=8) we found strong sst_{2A} expression in the inner plexiform layer (IPL) and moderate expression in the outer plexiform layer (OPL), the cellular membrane of the inner nuclear layer (INL) (Figure 8.1A), and the RPE. RPE stained most frequently at the apical side in a membranous pattern (Figure 8.1B), which was also noted in tangentially cut sections. Thick-walled choroidal vessels stained mostly positive, whereas chorio-capillaris only sporadically (Table 8.1). In negative controls, no staining was detected.

In the eyes with early ARM (n=3), sst_{2A} expression of the neuroretina, choroidal vessels and choriocapillaris was similar to normal controls (Table 8.1). The RPE stained positive in all cases. BLD were negative (Figure 8.1C).

In eyes with neovascular AMD (n=12), Bruch's membrane and BLD did not show sst_{2A} expression (Table 8.1). The choriocapillaris showed focal expression in only two eyes. Approximately 50 to 75% of thick-walled choroidal vessels stained positive, which was similar to normal controls. The CNV, both surgically excised and in enucleated eyes, could be subdivided in three groups, according to the activity of neovascularization. [120] The first group consisted of fibrovascular tissue with inflammatory cells, fibroblast-like cells and sparse fibrosis (n=2). The second group

consisted of fibrocellular scar tissue (n=2), and the third group consisted of a mixture of both fibrovascular and fibrocellular tissue (n=8). In the CNV, monolayers of pigmented cells adjacent to BLD were scored as RPE cells. Approximately half of these morphologically RPE cells showed sst_{2A} expression. The expression of sst_{2A} in newly formed endothelial cells was strong in fibrovascular membranes. Similarly, sst_{2A} was strongly expressed in endothelial cells of mixed fibrovascular and fibrocellular membranes (Figure 8.1D,E,F). Fibroblast-like cells and macrophages stained strongly positive in young membranes and less strongly in older scars (Figure 8.1D,E,F,G). Little or negative staining was observed in old hypocellular scars (Figure 8.1G). Expression of endothelial cells in fibrovascular membranes (61.5%) was found statistically significant more often than in fibrocellular membranes (29.5%; χ^2 analysis, $p<0.001$). Staining in CNV was considered specific, because peptide blocking significantly decreased staining of all structures mentioned.

In one eye with a mixed fibrovascular and fibrocellular membrane (eye number CNV10), we found positive staining of myofibroblasts in a hypercellular area of the underlying choroid in the posterior pole. This area also stained positive with antibodies directed against SMA and CD68, confirming the presence of myofibroblasts and macrophages.

In the eye with nonneovascular AMD, the staining pattern was similar to control tissue. The RPE stained positive. No staining was seen in the choriocapillaris, and vessels in the choroid were mostly positive.

RT-PCR

RT-PCR analysis of 3 posterior poles, including retina, RPE, choroid and sclera, revealed that mRNA encoding for sst_1, sst_{2A}, sst_3 and somatostatin is expressed in the posterior pole of normal human eyes. No mRNA encoding for sst_4 or sst_5 was detected (Figure 8.2, Table 8.2).

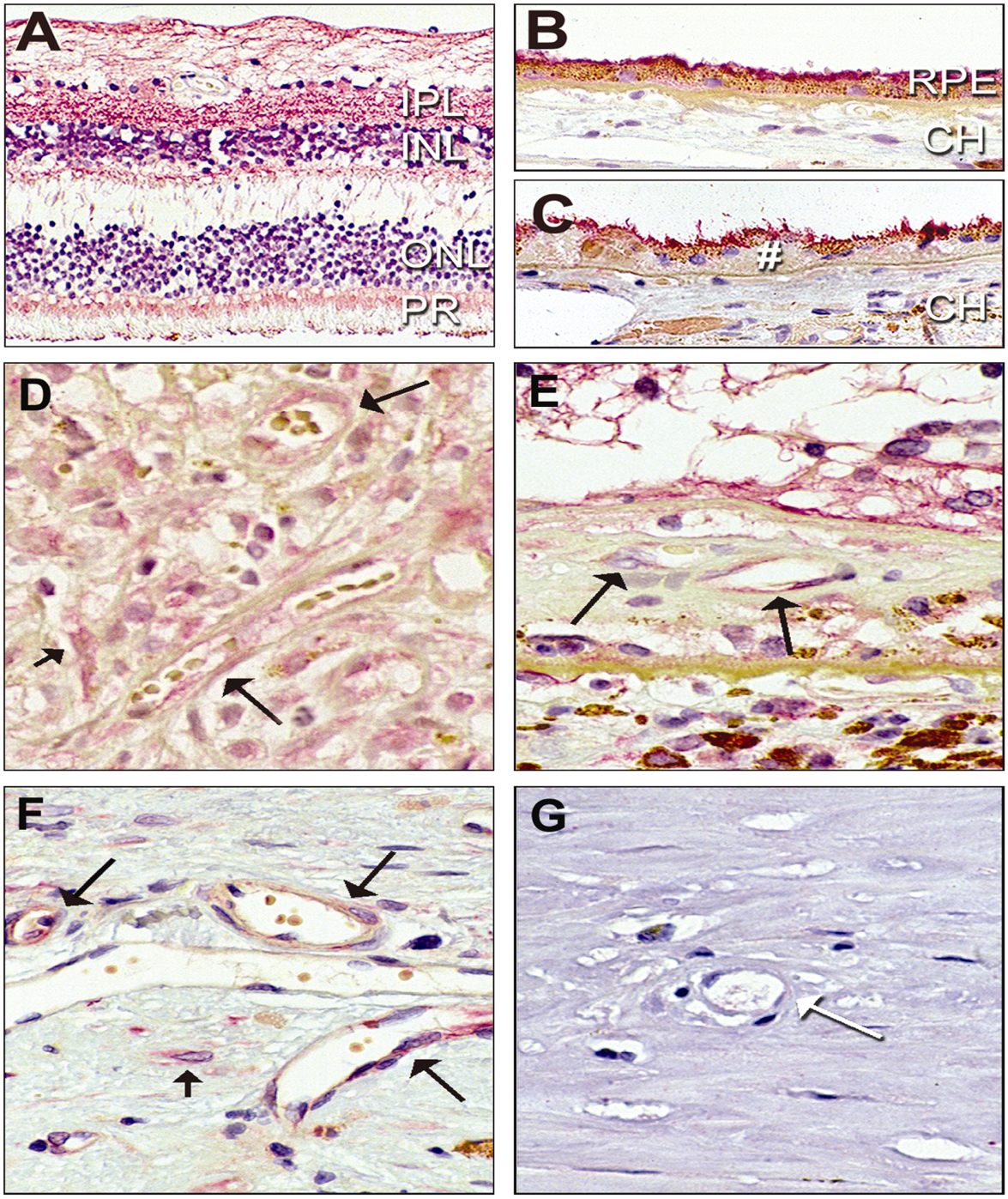

Figure 8.1 ***Immunolocalization of sst_{2A}*** *in posterior pole of normal eyes and eyes with different stages of ARM. Immunohistochemistry was performed on paraffin-embedded tissue, and visualized with an alkaline phosphatase detection system using a red chromogen.* ***(A)*** *Positive staining in normal neuroretina, with strong sst_{2A} expression in the inner plexiform layer (IPL) and moderate expression in the outer plexiform layer and the cellular membrane of the inner nuclear layer (INL).* ***(B)*** *sst_{2A} staining of normal RPE, showing the membranous staining pattern on the apical side.* ***(C)*** *sst_{2A} staining of an eye with early ARM, showing negative staining BLD and soft drusen (#).* ***(D through G)*** *sst_{2A} staining of CNV in eyes with ARM.* ***(D)*** *Surgically excised fibrovascular CNV (eye CNV1), with many positive fibroblast-like cells.* ***(E)*** *Fibrovascular CNV (eye CNV12).* ***(F)*** *Mixed fibrovascular and fibrocellular CNV (eye CNV11). Long arrows: positive endothelium of newly formed vessels; short arrows: positive fibroblast-like cells.* ***(G)*** *Staining of a fibrocellular CNV (eye CNV 13) with negative endothelial cells (white arrow) and fibroblast-like cells. ONL, outer nuclear layer; PR, photoreceptor layer; RPE, retinal pigment epithelium; CH, choroids; BM, Bruch's membrane; NR, overlying neuroretina. Original magnification* ***(A)*** *×200;* ***(B through G)*** *×400.*

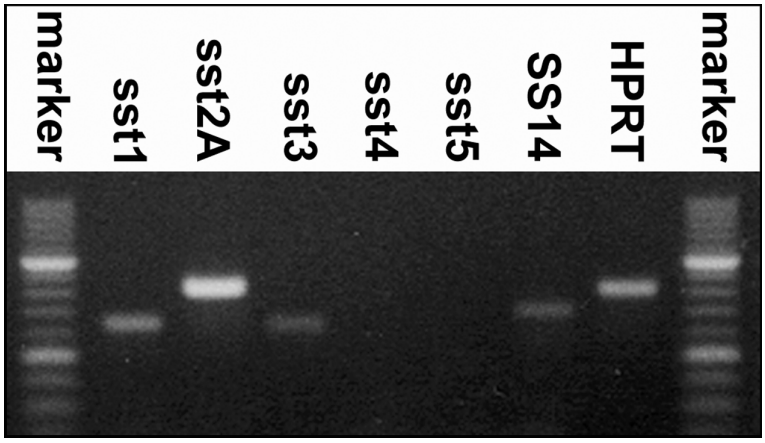

Figure 8.2 ***Expression of sst receptor subtype mRNA*** *in the posterior pole of a normal human eye, detected by RT-PCR. sst_1, sst_{2A}, and sst_3 were detected. Signals for sst_4 and sst_5 were too low to detect or absent. mRNA for somatostatin (SS14) was also detected. HPRT was used as internal control. Marker, 100 bp.*

DISCUSSION

In the present study normal human eyes and eyes with early and late stages of ARM express sst_{2A}. The localization of sst_{2A} expression in the neuroretina is consistent with findings in rabbit[292] and rat[293] retina and reflects the assumed physiological neuromodulator function of somatostatin.[299,300] In early stages of ARM, the choroidal vasculature and neuroretinal tissue stained identically with control tissue. We found no expression of sst_{2A} in BLD or drusen, which is in contrast with findings for other angiogenic growth factors such as VEGF.[144]

In eyes with neovascular AMD, we found strong expression of sst_{2A} in endothelial cells and fibroblast-like cells in early CNV. The expression of sst_{2A} in newly formed capillaries was abundant in fibrovascular CNV membranes. Similarly, in the active component of mixed fibrovascular/fibrocellular CNV, sst_{2A} was strongly expressed in endothelial cells. Grant and co-workers demonstrated the presence of somatostatin receptors on cultured human retinal endothelial cells.[173] They showed a direct inhibitory action of a somatostatin analogue on proliferation of these endothelial cells. Therefore, the angiogenic cells of CNV membranes may be capable of receiving angiogenic inhibition, directly receptor mediated or indirectly via inhibition of GH and IGF-I by somatostatin. In mice retina, somatostatin analogues have an inhibitory effect on neovascularisation.[177] Somatostatin analogues, such as the long-acting octreotide, which binds to somatostatin receptor subtypes 2 and 5, are used as experimental treatment in neovascular eye diseases such as diabetic retinopathy.[301-303]

We found strong sst_{2A} expression in fibroblast-like cells and macrophages in fibrovascular CNV and in intrachoroidal myofibroblasts. Sst_{2A} staining in

myofibroblasts may be due to cross-reactivity to myosin,[304] but macrophages have been shown to express sst_{2A}.[305] Macrophages and choroidal fibroblasts are thought to be one of the main sources of VEGF in the early stage of the disease.[155,247,290] Both macrophages and choroidal fibroblasts are also capable of releasing other angiogenic factors such as tumor necrosis factor-α (TNF-α) and IGF-I.[306] Somatostatin analogues have been shown to inhibit the release of macrophage and monocyte products such as TNF-α, interleukin (IL)-1β, IL-6 and IL-8 in vitro,[307,308] although there are also conflicting data.[309] The functional role of somatostatin with regard to the angiogenic factor synthesis and release has to be established.

In the overlying neuroretina of eyes with CNV, we found no obvious change of sst_{2A} expression and localization in comparison to normal eyes. This is in contrast to VEGF expression in neuronal tissue, which is upregulated under hypoxic circumstances.[144,148] This may indicate that the function of somatostatin on neuronal tissue is not influenced by hypoxic retinal disease. However, some care should be taken when interpreting these results, because they are semi quantitatively determined. It has recently been shown in a transgenic mice model that inhibition of GH, mediated by IGF-I, can inhibit ischemia-induced retinal neovascularization in vivo, but it does not reduce hypoxia-induced VEGF mRNA or protein levels. It was postulated that GH / IGF-I and VEGF may have distinct functions in the control of angiogenesis: VEGF may control acute oxygen regulation, whereas IGF-I may control neovascularization on the basis of availability of nutrients for cell division.[177] Our findings support the hypothesis that somatostatin and VEGF have distinct functions in the control of angiogenesis.

We confirmed local synthesis of sst_{2A} in the macula of normal human eyes with RT-PCR. We also demonstrated the expression of mRNA encoding for sst subtypes 1 and 3. In rats, sst_2 appeared to be the major subtype in the retina, but all other subtypes were expressed in retina and posterior pole as well.[294] Differential expression of sst has also been described previously in the immune system.[310] We also found mRNA expression of the neuropeptide somatostatin in the human macula. Production of somatostatin in the retina has been shown in rats with Northern blot hybridization and mRNA in-situ hybridization.[311-313] The production of both somatostatin and its receptors simultaneously suggests an autocrine action of somatostatin in the human retina.

From our findings we conclude that the sst_{2A} receptor in choroid and retina of early ARM and nonneovascular AMD is localized similar to normal controls. In eyes with CNV, the sst_{2A} receptor is strongly expressed in the fibrovascular phase of CNV, as well as in intrachoroidal myofibroblasts. mRNA of sst subtypes 1, 2A and 3, as well as mRNA of somatostatin are expressed in the macula of the normal human eye. The functional role of somatostatin with regard to the synthesis and release of angiogenic

factors has to be established. Because of the sst expression in CNV, somatostatin analogues may be an effective therapy in early stages of neovascular AMD.

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