Somatostatin Receptor 2A Expression in Choroidal Neovascularization Secondary to Age-Related Macular Degeneration

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PURPOSE. The growth of ocular neovascularization is regulated by a balance between stimulating and inhibiting growth factors. Somatostatin affects angiogenesis by inhibiting the growth hormone-insulin-like growth factor axis and also has a direct antiproliferative 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 (sst2A) in normal human macula, and to study sst2A 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 sst2A antibody. mRNA expression of five ssts and somatostatin was determined in the posterior pole of three normal human eyes by reverse transcriptase-polymerase chain reaction.

RESULTS. The immunohistochemical expression of sst2A in newly formed endothelial cells and fibroblast-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 sst2A. The presence of mRNA of sst subtype 2A was observed in normal human macula, and subtypes 1 and 3 and somatostatin are also present. SST2A receptors bind potential anti-angiogenic somatostatin analogues such as octreotide. Therefore, somatostatin analogues may be an effective therapy in early stages of CNV in ARM. (Invest Ophthalmol Vis Sci. 2000;41:2329–2335)

Ag-related maculopathy (ARM) is the major cause of blindness in people more than 65 years of age in the Western world. The prevalence of ARM is up to 14% in people aged more than 85 years.1 Late stages of ARM, also called age-related macular degeneration (AMD), include geographic atrophy and exudative macular degeneration. The exudative form is characterized by choroidal neovascularization (CNV) and is responsible for 80% of cases of severe vision loss.1 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.2 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.2 Vascular endothelial growth factor (VEGF), an endothelium-specific mitogen, is regarded as one of the most important ocular angiogenic factors, especially in ischemic disease.2–8 Other regulating growth factors include fibroblast growth factors (FGFs), transforming growth factor (TGF)-β and insulin-like growth factor (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.4,5,9–13

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.14 Somatostatin binds with high affinity to five subtype receptors (sst types 1 to 5). These receptors were identified in various animal retinas.15–17 The exact role of a direct receptor-mediated effect by somatostatin analogues is still unknown. To date, information about sst2 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 sst<sub>2A</sub> 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 of 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 1. Eight eyes (of seven patients) had clinical diagnoses of AMD. In eight other eyes, ARM was diagnosed histopathologically according to the following criteria: Early stages of ARM (< 5) were characterized by the presence of basal laminar deposits, basal linear deposits (BLD), soft drusen, and thickening of Bruch’s membrane. Exudative AMD (< 12) was classified as sub-RPE CNV, subretinal CNV (between neuroretina and RPE) or mixed sub-RPE and subretinal CNV. Photoreceptors, Bruch’s membrane, and BLD were helpful in the orientation of the specimens. 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.

In CNV, we recorded the presence of fibrovascular or fibrocellular scar; BLD, basal laminar deposits; HEM, hemorrhage; RPE, retinal pigment epithelium; CC, choriocapillaris; CH, choroidal vessels; CNV, choroidal neovascularization; EC, endothelial cells; FBL, fibroblast-like cells; U, unknown; NC, not classifiable; NP, not present; NA, not applicable.

† sst<sub>2A</sub> expression in endothelial cells in CNV was quantitatively determined by counting the proportion of positive vessels in randomly selected sections.

**Immunohistochemistry**

Rabbit antihuman sst<sub>2A</sub> polyclonal antibody (R2-88) was kindly provided by Agnes Schonbrunn (Department of Integrative Biology and Pharmacology, University of Texas Houston Medical School). The antibody was raised against a 22-amino acid peptide located at the C-terminal region of the sst<sub>2</sub> receptor. The sst<sub>2A</sub> antibody had been characterized and tested before by Western blot analysis and peptide binding. The sst2A antibody had been characterized and tested before by Western blot analysis and peptide binding.
Somatostatin Receptors in Choroidal Neovascularization

**TABLE 2.** Patient Data and sst Receptor Subtype Expression in Normal Eyes

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Eye</th>
<th>Clinical Description</th>
<th>sst Receptor Subtype Expression* (RT-PCR)</th>
<th>sst Receptor Subtype Expression† (Immunohistochemistry)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>sst₁</td>
<td>sst₂A</td>
</tr>
<tr>
<td>1 71/U</td>
<td>OD</td>
<td>Donor eye</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2 51/M</td>
<td>OD</td>
<td>Ciliary body melanoma</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3 78/M</td>
<td>OS</td>
<td>Choroidal melanoma</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4 81/M</td>
<td>OS</td>
<td>Tarsal squamous cell carcinoma</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5 42/M</td>
<td>OS</td>
<td>Choroidal melanoma</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>6 76/F</td>
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<td>Choroidal melanoma</td>
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<td>ND</td>
</tr>
<tr>
<td>7 57/M</td>
<td>OS</td>
<td>Recurrent conjunctival melanoma</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8 60/M</td>
<td>OS</td>
<td>Choroidal melanoma</td>
<td>ND</td>
<td>ND</td>
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<td>9 69/M</td>
<td>OD</td>
<td>Ciliary body adenoma</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 78/M</td>
<td>OS</td>
<td>Spindle cell nevus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11 26/M</td>
<td>OS</td>
<td>Choroidal melanoma</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

sst₁, somatostatin; HPRT, hypoxanthine-guanine phosphoribosyl transferase; RPE, retinal pigment epithelium; CC, choriocapillaris; CH, choroidal vessels. U, unknown; ND, not done.

* Categories of sst subtype expression (RT-PCR): = no expression, + = positive expression.

† Categories of sst₂A expression (immunohistochemistry): 0 = 0–10% positive cells; + = 11%–50% positive cells; + + = 51%–100% positive cells.

RESULTS

Immunohistochemistry

In normal retina (n = 8) we found strong sst₂A expression in the inner plexiform layer and moderate expression in the outer plexiform layer, the cellular membrane of the inner nuclear layer (Fig. 1A), and the RPE. RPE stained most frequently at the apical side in a membranous pattern (Fig. 1B), which was also noted in tangentially cut sections. Thick-walled choroidal vessels stained mostly positive, but choriocapillaris only sporadically (Table 1). In negative controls, no staining was detected.

In the eyes with early ARM (n = 5), sst₂A expression of the neuroretina, choroidal vessels, and choriocapillaris was similar to normal controls (Table 1). The RPE stained positive in all cases. BLD were negative (Fig. 1C).

In eyes with exudative AMD (n = 12), Bruch’s membrane and BLD did not show sst₂A expression (Table 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. 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 \((n = 8)\).19

In the CNV, monolayers of pigmented cells adjacent to BLD were scored as RPE cells. Approximately half of these morphologically RPE cells showed sst2A expression. The expression of sst2A in newly formed endothelial cells was strong in fibrovascular membranes. Similarly, sst2A was strongly expressed in endothelial cells of mixed fibrovascular and fibrocellular membranes (Fig. 1E, 1F, 1G). Fibroblast-like cells and macrophages stained strongly positive in young membranes and less strongly in older scars (Fig. 1E, 1G, 1H). Little or no staining was observed in old hypocellular scars (Fig. 1E, 1F, 1G, 1H). Expression in endothelial cells of mixed fibrovascular and fibrocellular membranes (61.5%) was statistically significant more often than in fibrovascular membranes (29.5%; \(\chi^2\) analysis, \(P < 0.001\)). Staining in endothelial cells in fibrovascular membranes (61.5%) was statistically significant more often than in fibrovascular membranes (29.5%; \(\chi^2\) analysis, \(P < 0.001\)). Staining in endothelial cells in fibrovascular membranes (61.5%) was statistically significant more often than in fibrovascular membranes (29.5%; \(\chi^2\) analysis, \(P < 0.001\)). Staining in endothelial cells in fibrovascular membranes (61.5%) was statistically significant more often than in fibrovascular membranes (29.5%; \(\chi^2\) analysis, \(P < 0.001\)). Staining in endothelial cells in fibrovascular membranes (61.5%) was statistically significant more often than in fibrovascular membranes (29.5%; \(\chi^2\) analysis, \(P < 0.001\)). Staining in endothelial cells in fibrovascular membranes (61.5%) was statistically significant more often than in fibrovascular membranes (29.5%; \(\chi^2\) analysis, \(P < 0.001\)).

In one eye with a mixed fibrovascular and fibrocellular membrane (eye 12), we found positive staining of myofibroblasts in a hypercellular area of the underlying choroid in the posterior pole. This area also stained positively 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 positively. No staining was seen in the choriocapillaris, and vessels in the choroid were mostly positive.

**Reverse Transcriptase-Polymerase Chain Reaction**

RT-PCR analysis of three posterior poles, including retina, RPE, choroid, and sclera, revealed that mRNA encoding for sst1, sst2A, sst3, and somatostatin is expressed in the posterior pole of normal human eyes. No mRNA encoding for sst4 or sst5 was detected (Fig. 2, Table 2).

### DISCUSSION

In the present study normal human eyes and eyes with early and late stages of ARM expressed sst2A. The localization of sst2A expression in the neuroretina is consistent with findings in rabbit15 and rat16 retina and reflects the assumed physiological neuromodulator function of somatostatin.24,25 In early stages of ARM, the choroidal vasculature and neuroretinal tissue stained identically with control tissue. We found no expression of sst2A in BLD or drusen, which is in contrast with findings for other angiogenic growth factors such as VEGF.3

In eyes with exudative AMD, we found strong expression of sst2A in endothelial cells and fibroblast-like cells in early CNV. The expression of sst2A in newly formed capillaries was abundant in fibrovascular CNV membranes. Similarly, in the active component of mixed fibrovascular–fibrocellular CNV, sst2A was strongly expressed in endothelial cells. Grant et al.26 demonstrated the presence of somatostatin receptors on cultured human retinal endothelial cells. 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 through inhibition of GH and IGF-I by somatostatin. In mice retina, somatostatin analogues have an inhibitory effect on neurovascularization.14 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.27–29

We found strong sst2A expression in fibroblast-like cells and macrophages in fibrovascular CNV and in intrachoroidal myofibroblasts. sst2A staining in myofibroblasts may be due to cross-reactivity to myosin,30 but macrophages have been shown to express sst2A.31 Macrophages and choroidal fibroblasts are thought to be one of the main sources of VEGF in the early stage of the disease.6,10,52 Both macrophages and choroidal fibroblasts are also capable of releasing other angiogenic factors such as tumor necrosis factor (TNF)-a and IGF-I.53 Somatostatin analogues have been shown to inhibit the release of inflammatory mediators such as tumor necrosis factor (TNF)-a and IGF-I.53

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Primer</th>
<th>Sequence (5′–3′)</th>
<th>Product Size (base pair)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sst1</td>
<td>Forward</td>
<td>ATGCGTGGCCCTCAAGGGCGGG</td>
<td>318</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TGGGGGGATAGGTAAGAAGGAA</td>
<td>414</td>
</tr>
<tr>
<td>sst2A</td>
<td>Forward</td>
<td>GCCAGAGTGAAAGCAGATGAC</td>
<td>314</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GTAGAGCCCTTGTTACGAGCAAGC</td>
<td>321</td>
</tr>
<tr>
<td>sst3</td>
<td>Forward</td>
<td>GCCACCTGCTATCCCTACAAC</td>
<td>226</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CGGTCTTATCTATCGAAGG</td>
<td>349</td>
</tr>
<tr>
<td>SST4</td>
<td>Forward</td>
<td>GATGCTGCTCCGCGCCTCCAG</td>
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<tr>
<td></td>
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<td>ATGGTGGCCCTCAAGGCCGG</td>
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</tr>
<tr>
<td>HPRT</td>
<td>Forward</td>
<td>CAGGACTGAACTGCTGTGCTG</td>
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<td>Reverse</td>
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<tr>
<td></td>
<td>Reverse</td>
<td>CAGGATGTCAGATCGAAGG</td>
<td>314</td>
</tr>
</tbody>
</table>

The sequences of the primers for sst1 were derived and adapted from Wulfson et al.41 for sst2A 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.
of macrophage and monocyte products such as TNF-α, interleukin (IL)-1β, IL-6 and IL-8 in vitro, although there are also conflicting data. 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 sst2A expression and localization in comparison to normal eyes. This is in contrast to VEGF expression in neuronal tissue, which is upregulated under hypoxic circumstances. 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 semiquantitatively determined. It has recently been shown in a transgenic mouse 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

**FIGURE 2.** Expression of sst receptor subtype mRNA in the posterior pole of a normal human eye, detected by RT-PCR. sst1, sst2A, and sst3 were detected. Signals for sst4 and sst5 were too low to detect or absent. mRNA for somatostatin (SS14) was also detected. HPRT was used as internal control. Marker, 100 bp.
has been postulated that GH-IGF-I and VEGF 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.13 Our findings support the hypothesis that somatostatin and VEGF have distinct functions in the control of angiogenesis.

We confirmed local synthesis of sst2A 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, sst2 appeared to be the major subtype in the retina, but all other subtypes were expressed in retina and posterior pole as well.17 Differential expression of sst has also been described previously in the immune system.37 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 analysis hybridization and mRNA in situ hybridization.38–40 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 sst2A receptor in choroid and retina of early ARM and nonneovascular AMD is localized similar to normal controls. In eyes with CNV, the sst2 receptor is strongly expressed in the fibrovascular phase of CNV, as well as in intrachorioidal 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 CNV in AMD.

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**References**


