Expression, Localization, and Functional Coupling of the Somatostatin Receptor Subtype 2 in a Mouse Model of Oxygen-Induced Retinopathy

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PURPOSE. In the mouse model of oxygen-induced retinopathy (OIR), somatostatin-14 (SRIF) acting at the SRIF receptor subtype 2 (sst2) inhibits angiogenic responses to hypoxia through a downregulation of vascular endothelial growth factor. Information about where SRIF-sst2 interactions take place is lacking, and downstream effectors mediating SRIF-sst2 antiangiogenic actions are unknown.

METHODS. In the OIR model, retinal expression of SRIF was evaluated with RT-PCR and radioimmunoassay. The bindings of [125I]LT3-SRIF-28 and [125I]Tyr3-octreotide were measured in coronal sections of the eye. With Western blot analysis, the authors evaluated the levels of sst2A and the expression and activity of the signal transducer and activator of transcription (STAT3). The analysis of STAT3 was performed in hypoxic mice treated with the sst2 agonist octreotide or with the sst2 antagonist D-Tyr8 cyanamid 154806 (CYN). Retinal localization of sst2A was assessed by single and double immunohistochemistry with an endothelial cell marker.

RESULTS. In the hypoxic retina, both SRIF and sst2 levels as well as [125I]Tyr3-octreotide binding were downregulated. In addition, sst2A immunostaining was decreased in the neuroretina but was increased in capillaries. Hypoxia increased both the expression and the activity of STAT3. This increase was inhibited by octreotide but was strengthened by CYN.

CONCLUSIONS. These data suggest that sst2 expressed by capillaries may be responsible for the antiangiogenic effects of SRIF and that downstream effectors in this action include the transcription factor STAT3. These results support the possibility of using sst2-selective ligands in the treatment of proliferative retinopathies and indicate STAT3 as an additional target for a novel therapeutic approach. (Invest Ophthalmol Vis Sci. 2010; 51:1848–1856) DOI:10.1167/iovs.09-4472
also increased in a mouse model of retinal ischemia. More- 
over, STAT3 phosphorylation is increased in retinal macro-
phages from OIR mice. There are also results demonstrating that STAT3 is crucial to modulate VEGF expression and activity in vascular endothelial cells, thus indicating a possible role of STAT3 in regulating angiogenic response to the hypoxic in-

In the present study, we determined whether low oxygen avail-
ability may affect levels and localization of sst2 in the mouse retina and whether this effect may be related to altered levels of SRIF expression. In addition, we evaluated whether hypoxia affects the expression and activity of STAT3 and whether the effects of hypoxia on STAT3 are influenced by treatment with the sst2 agonist octreotide or the sst2-selective antagonist CYN.

**Materials and Methods**

Octreotide was purchased from NeoMPS (Strasbourg, France). Super-
mix (IQ Sybr Green) was from Bio-Rad (Hercules, CA). Primers were obtained from Eurofins MWG Operon (Ebersberg, Germany). Nucleic acid stain (GelStar) was from Cambrex (East Rutherford, NJ). LTT-

SRIF-28 and Tyr-3-octreotide were obtained by Peninsula Laboratories (Meyerside, UK) and GenScript Corporation (Piscataway, NJ), respectively. A rabbit polyclonal antibody directed to sst2 was purchased from Gramsch Laboratories (Schwabhausen, Germany). In addition, a rabbit monoclonal antibody directed to CD31 was purchased from BD PharMingen (San Diego, CA). A rabbit polyclonal antibody to tyrosine hydroxylase (TH) was obtained from Chemicon (Temecula, CA). Appropriate secondary antibodies were from Molecular Probes (Eugene, OR). Rabbit polyclonal antibodies directed to STAT3 and pSTAT3 were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). The enhanced chemiluminescence reagent WBKLS0500 was from Millipore (Billerica, MA). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

**Animals**

Experiments were performed on 68 mice (C57BL/6) of both sexes at postnatal day (PD) 17 (6 g body weight). In some experiments, mice at PD12 and PD14 were also used (15 animals for each age). Experiments were performed in agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and in compliance with the Italian law on animal care no.116/1992 and the EEC/609/86. All efforts were made to reduce the number of animals used.

**Model of Oxygen-Induced Retinopathy**

In a typical model of OIR, litters of mice pups with their nursing mothers were exposed in an infant incubator to high oxygen concentra-
tion (75% ± 2%) between PD7 and PD12, prior to return to room air between PD12 and PD17. Oxygen was checked twice daily with an oxygen analyzer (Miniox I; Bertocchi srl Elettromedicali, Cremona, Italy). Individual litters were either oxygen or room air reared. In some experiments, pharmacologic treatment was also performed, including no treatment, treatment with SRIF analogues, and sham injection. Animals were treated with injections for 5 days from PD12 to PD16. Animals were anesthetized by intraperitoneal injection of 1.2% tribromoethanol and 2.4% amylene hydrate in distilled water (0.02 ml/g body weight; Avertin, Pierce, Rockford, IL). All experiments were performed at the same time of day to exclude possible circadian influences. The data were collected from both males and females, and the results were combined because there was no apparent gender difference.

**Administration of SRIF Analogues**

The sst2-prefering peptidyl agonist octreotide and the sst2-selective peptidyl antagonist CYN19–21 were given twice daily subcutaneously from PD12 to PD16 at 0.02 mg/kg per dose (octreotide) or at 0.5 mg/kg per dose (CYN), as previously described. The analogues were dis-

olved in 33 mM acetate buffer (pH 5) with 135 mM NaCl. Sham injections were performed with that vehicle.

**Isolation of Retinal RNA and cDNA Preparation**

Total RNA was extracted from explanted retinas (RNeasy Mini Kit; Qiagen, Valencia, CA), purified, resuspended in RNase-free water, and quantified spectrophotometrically (SmartSpec 3000; Bio-Rad). First-

strand cDNA was generated from 1 μg total RNA (QuantiTect Reverse Transcription Kit; Qiagen).

**Real-Time Quantitative RT-PCR**

Real-time quantitative RT-PCR (QPCR) was performed according to Dal Monte et al.5 SRIF primers (forward, CCCCAGACTCCGTCAAGTTCT; reverse, TCTCTGTCGGTGCGTCCTG) were designed using Primer3 software,5 whereas primer pairs for Rpl13a (forward, CACTCTGGAG-

GAGAAACGGAAGG; reverse, GCAGGCTAGGGCCAAACATGCT) were obtained from RTPriimerDB. Amplification efficiency was close to 100% for both primer pairs, as calculated (Opticon Monitor 3 software; Bio-Rad). SRIF target gene was run concurrently with Rpl13a, a con-

stitutively expressed control gene. As previously described, samples were compared using the relative cycle threshold (CT method).24 The increase or decrease (x-fold) was determined relative to a control after normalizing to Rpl13a. All reactions were run in triplicate. After statis-

tical analysis, the data from the different experiments were plotted and averaged in the same graph. Data are expressed as mean ± SE and originated from four samples for either control or hypoxic condition. Each sample refers to the mRNA extracted from three retinas.

**SRIF Measurements**

Explanted retinas were homogenized in 10 wt/vol acetic acid (2 N). SRIF levels were evaluated by radioimmunoassay, as previously de-

scribed. Results of the radioimmunoassay are normalized for the amount of protein per retina and are expressed as mean ± SE from six retinas for either control or hypoxic conditions.

**Autoradiography**

For SRIF receptor autoradiography, LTT-SRIF-28 and Tyr-3-octreotide were iodinated as previously described26 and used at a specific activity of 422 Ci/mmol and 662 Ci/mmol, respectively. Eyes from three control and three hypoxic mice were enucleated, quickly frozen in liquid nitrogen, and stored at −80°C. Sections (16 μm) were cut on a cryostat. Receptor autoradiography was performed as previously de-

scribed. The sections were incubated in 545 pM [125I]LTT-SRIF-28 or 260 pM [125I]Tyr-3-octreotide. Nonspecific binding was determined in a set of adjacent slides by incubation in the presence of 1 μM SRIF-14. Autoradiograms were generated by applying the labeled sections to films (BioMax MR; Kodak, Rochester, NY) at 4°C for 5 days. Photomi-
crographs of films were generated and analyzed using a computerized image analysis system with the Mercator software (BIOCOM; Explora Nova-Mercator, La Rochelle, France). Autoradiographic quantification was expressed as pmol/retina surface, and specific binding was calcu-

lated as pmol/retina surface area unit assessed in total binding − pmol/retina surface area unit assessed in nonspecific binding. Data are expressed as mean ± SE and originated from six retinas for either control or hypoxic conditions.

**Western Blot Analysis**

Western blot analysis was performed on proteins extracted from three samples for each experimental condition, according to Dal Monte et al. Samples from age-matched control subjects were used for compar-

ison. Each sample contained five retinas. Western blot analysis for STAT3 and pSTAT3 was performed on cytosolic proteins, extracted in buffer containing 0.1 M sodium orthovanadate, 20 mM βglycerophosphate, and 20 mM p-nitrophenylphosphate. Western blot analysis for sst2A was performed on the fraction containing membrane-bound
proteins. Aliquots of each sample containing equal amounts of protein were subjected to SDS-PAGE on 10% acrylamide gels. β-Actin was used as the loading control. Rabbit polyclonal antibodies directed to STAT3 (1:200 dilution) or sst2A (1:500 dilution) or mouse monoclonal antibodies directed to pSTAT3 (1:200 dilution) or β-actin (1:2500 dilution) were used as primary antibodies. Mouse anti-rabbit horseradish peroxidase–labeled (1:5000 dilution) or rabbit anti–mouse horseradish peroxidase–labeled (1:25,000 dilution) antibodies were used as secondary antibodies. Blots were developed with the enhanced chemiluminescence reagent, stripping them in between each assay. All experiments were run in duplicate. The semiquantitative analysis of Western blot signals was based on three independent blot-analysis experiments. After statistical analysis, data from the different experiments were plotted and averaged in the same graph.

**Immunohistochemistry**

Eyes were removed and immersion fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 1 hour. Then the fixed eyes were transferred to 25% sucrose in 0.1 M PB and stored at 4°C. Retinal sections were cut perpendicularly to the vitreal surface at 10 μm with a cryostat, mounted onto gelatin-coated slides, and stored at -20°C. Rabbit antiserum against the sst2A isoform was used at 1:500 dilution. In addition, a rat monoclonal antibody directed to the well-known endothelial cell marker CD31 was used at 1:200 dilution for the detection of retinal vessels. Finally, a mouse monoclonal antibody to protein kinase C (PKC) was used at 1:200 to label rod bipolar cells, and a rabbit polyclonal antibody to TH was used at 1:400 to label distinct wide-field amacrine cells. The sections were incubated with the appropriate secondary antibody conjugated with Alexa Fluor 488 or Alexa Fluor 546 at a dilution of 1:200. Control experiments included the omission of the primary antibodies. Unspecific staining was not observed. In double-labeling studies, control experiments were also performed to ensure that the primary antibodies did not cross-react when mixed together and that the secondary antibodies reacted only with the appropriate antigen-antibody complex. Immunofluorescence images were acquired using a 40 × plan Zeiss objective (NEOFLUAR; Carl Zeiss Vision GmbH, München-Hallbergmoos, Germany), an Axioscam camera, and Zeiss software (Axiovision 4; Carl Zeiss Vision GmbH). The digital images were sized and optimized for contrast and brightness using image editing software (Photoshop; Adobe Systems, Mountain View, CA). Final images were saved at a minimum of 300 dpi.

Quantitative analysis of double-labeled retinal sections was performed in agreement with Catalani et al. Briefly, both the CD31- and the sst2A-immunoreactive images relative to the same field were visualized. Subsequently, the images were turned into grayscale, normalized to the background, and thresholded to obtain white immunostaining on a black background. Using Zeiss software (S-300; Carl Zeiss Vision GmbH), the area of CD31 immunostaining and that of sst2A-immunoreactivity (IR) were calculated. Finally, the ratio between the area covered by the sst2A staining and that covered by the CD31-IR was calculated and used as an index of the overlap area. Data are expressed as mean ± SE and originated from six retinas for either control or hypoxic conditions.

**Statistical Analysis**

All data were analyzed by the Kolmogorov-Smirnov test on verification of normal distribution. Statistical significance was evaluated with unpaired t-test or with ANOVA followed by the Newman-Keuls multiple comparison test. Results are expressed as mean ± SE of the indicated n values (Prism; GraphPad Software, San Diego, CA). Differences with P < 0.05 were considered significant.

**RESULTS**

**Hypoxia Effects on SRIF**

As shown in Figure 1A, RT-PCR yielded amplified products at 110 bp corresponding to SRIF mRNA. Five days of normoxia

**Figure 1.** Retinal levels of SRIF in hypoxic mice. (A) PCR products of SRIF mRNA (110 bp) and the housekeeping gene Rpl13a (182 bp) in normoxic control retinas. (B) SRIF mRNA in control (white) and hypoxic (black) conditions. QPCR evaluation showed that SRIF messenger was not influenced by hypoxia. Data were analyzed by the formula 2^{-ΔΔCT} using Rpl13a as an internal standard. Each column represents the mean ± SE of data from four samples. Each sample refers to the mRNA extracted from three retinas. (C) Endogenous level of SRIF in the retinas of control (white) and hypoxic (black) mice. Radioimmunoassay measurement showed that SRIF level was decreased by hypoxia (*P < 0.05 vs. control; unpaired t-test). The amount of SRIF is expressed as picogram per milligram of proteins. Each column represents the mean ± SE of data from six retinas.
after hyperoxia (relative hypoxia) did not influence the amount of SRIF mRNA, which was similar to that in control retinas (Fig. 1B). In contrast, as shown in Figure 1C, SRIF levels measured by radioimmunoassay were significantly decreased by hypoxia (~65% lower than in control retinas; *P* < 0.05).

**Hypoxia Effects on sst2 Binding and sst2A Expression**

The data summarized in Table 1 show high levels of SRIF binding sites in mouse retinas, with radioligand concentrations in the picomolar range. Nonspecific binding was low for both radioligands used. In hypoxic retinas, [125I]LTT-SRIF-28 binding sites were not significantly different from the respective values in control retinas. In contrast, [125I]Tyr3-octreotide binding sites were significantly decreased by ~30% (Fig. 2; Table 1).

Regarding the levels of sst2A, semiquantitative Western blot analysis showed that they were not significantly different from control values both at PD12 (end of the period of hypoxia) and at PD14 (2 days of normoxia). In contrast, 5 days after hypoxia (relative hypoxia, PD17), retinal levels of sst2A were significantly lower than those in control conditions (~26%; 

**Hypoxia Effects on sst2A Localization**

Immunostaining patterns of sst2A in control retinas were consistent with previous observations of the mouse retina. In particular, sst2A-IR was localized primarily to rod bipolar cells and to amacrine cells, whereas it was scarcely associated with retinal capillaries labeled with CD31 (Figs. 4A, 4C). Hypoxia caused a drastic reduction of sst2A immunostaining in retinal neurons accompanied by an evident increase of sst2A-IR in retinal blood vessels (Figs. 4B, 4D). Retinal vasculature includes three layers of capillary networks: the most superficial layer of capillaries in the inner part of the nerve fiber layer (NFL), the inner capillaries in the ganglion cell layer (GCL), and the outer capillary network from the inner plexiform layer (IPL) to the outer plexiform layer (OPL) through the inner nuclear layer (INL). As shown in Figure 4E, the mean overlap area covered by sst2A-IR and CD31-IR almost doubled in the IPL-INL of hypoxic retinas compared with control retinas. In contrast, hypoxia did not affect the mean overlap area in the NFL-GCL. Additional experiments were performed to determine whether hypoxia-induced decreases of sst2A-IR might result from hypoxia-induced alterations of the retinal cells, which are known to express sst2A. In the rodent retina, sst2A is in TH-containing amacrine cells and in rod bipolar cells, as identified by PKC-IR. As shown in Figure 5, hypoxia did not influence TH or PKC immunostaining patterns.

**Hypoxia Effects on STAT3**

As shown in Figure 6, STAT3 was significantly increased in the hypoxic samples compared with controls (60%; *P* < 0.001).

**TABLE 1.** Autoradiographic Analysis of SRIF Binding Sites from both Control and Hypoxic Mouse Retinas

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>Specific Binding</th>
<th>Hypoxic</th>
</tr>
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<tbody>
<tr>
<td>[125I]LTT-SRIF-28</td>
<td>3.91 ± 0.16</td>
<td>3.67 ± 0.28</td>
</tr>
<tr>
<td>[125I]Tyr3-octreotide</td>
<td>3.51 ± 0.26</td>
<td>2.83 ± 0.08*</td>
</tr>
</tbody>
</table>

* Data are expressed as specific binding (pmol/retina surface area unit assessed in total binding – pmol/retina surface area unit assessed in nonspecific binding) ± SE of 12 to 17 coronal sections.

* *P* < 0.05 vs. the respective control (unpaired *t*-test).

**DISCUSSION**

The results of this study demonstrate for the first time that in the OIR model hypoxia upregulates sst2A expression by retinal vessels, suggesting that the growing endothelium overexpresses sst2A and becomes capable of efficiently responding to the angiinhibitory action of SRIF. The present findings also indicate that hypoxia upregulates STAT3 expression and activ-
ity in the OIR model and suggest that STAT3 plays an important role in mediating sst2 action in proliferative retinopathy.

Hypoxia Effects on SRIF

As shown herein, retinal levels of SRIF are downregulated in the OIR model. This result is consistent with the decreased SRIF levels observed in the vitreous and in the retinas of diabetic patients, and it demonstrates that the ocular deficit of SRIF may contribute to the onset of diabetic retinopathy. In this respect, protective effects of SRIF and its analogues have been demonstrated recently in rodent models of retinal neurodegeneration.

The fact that, after hypoxia, endogenous SRIF decreases in the retina but SRIF mRNA does not change suggests a downregulation at the posttranscriptional level involving translation pathways that do not interact with nuclear regulatory factors. This possibility is consistent with previous findings suggesting the involvement of posttranslational mechanisms in the regulation of SRIF expression in the retina of mice with genetic deletions of the SRIF receptor sst1 or sst2.

Hypoxia Effects on sst2

Our results show that hypoxia downregulates retinal levels of the sst2A isoform of sst2, suggesting that sst2A downregulation is associated with the onset of angiogenesis in the retina of the OIR mouse model. That hypoxia might regulate the expression of cell surface receptors has been demonstrated, in different experimental models, for the adenosine receptors of the A2A subtype, the delta opioid receptors, and the glucocorticoid receptors. However, to the best of our knowledge, no results are available reporting hypoxia-induced regulation of SRIF receptors. As shown herein, sst2A reduction is not accompanied by changes in sst2 gene expression, as demonstrated by previous findings, indicating the involvement of translational or posttranslational mechanisms.

Our autoradiographic studies confirmed the decrease of sst2 protein levels in hypoxic retinas and showed a marked decrease in [125I]Tyr3-octreotide binding sites. Although
[125I]Tyr3-octreotide labels both recombinant sst2 and sst5 with high affinity, the observed binding is likely to be due entirely to the presence of sst2. Because [125I]LTT-SRIF-28 labels all SRIF receptors with high affinity, the fact that in control retinas [125I]LTT-SRIF-28 binding sites have a density similar to that in hypoxic retinas suggests that the total number of SRIF receptors does not change as a consequence of hypoxia and implies that the decrease of sst2 after hypoxia is compensated by an increase in the levels of other SRIF receptors. Indeed, a major effect of sst2 loss on the expression of sst1 has been demonstrated previously in the mouse retina. The mechanisms leading to reduced sst2 levels in hypoxic retinas remain to be elucidated. It is likely that SRIF, sst1, and sst2 cooperate in complex regulatory mechanisms controlling their own expressions in the retina (see Ref. 3 for a discussion).

Our previous observations indicate a strict correlation between retinal SRIF levels and sst2 expression, but at present it is difficult to determine whether an effect of hypoxia on SRIF retinal levels would, in turn, affect sst2 expression or, conversely, whether an effect of hypoxia on sst2 would affect SRIF levels in the retina. On the other hand, in the mouse retina, the possibility that SRIF levels are regulated by sst2 seems unlikely.

![FIGURE 5](image-url)  
**FIGURE 5.** Retinal sections showing TH (A, B) and PKC (C, D) immunolabeling in control (A, C) and hypoxic (B, D) conditions. Scale bar, 20 μm.

![FIGURE 6](image-url)  
**FIGURE 6.** Levels of STAT3 (A) and pSTAT3 (B) in hypoxic retinas after treatment with vehicle, 0.02 mg/kg octreotide or 0.5 mg/kg CYN, as evaluated by Western blot analysis using β-actin as the loading control. (A, B) Densitometric analysis showed that both STAT3 and pSTAT3 were increased by hypoxia (*P < 0.001 vs. the respective control; ANOVA followed by Newman-Keuls multiple comparison posttest). STAT3 level in vehicle-treated mice was not significantly different from that in hypoxic mice. The hypoxia-induced increase in both STAT3 and pSTAT3 was significantly lowered by octreotide (§§P < 0.001 vs. the respective vehicle-treated; ANOVA followed by Newman-Keuls multiple comparison posttest). The hypoxia-induced increase of STAT-3 was unaffected by CYN, whereas the hypoxia-induced increase in pSTAT3 was increased by CYN (§P < 0.05 vs. the respective vehicle-treated; ANOVA followed by Newman-Keuls multiple comparison posttest). Each column represents the mean ± SE of data from three samples. Each sample refers to the protein extracted from five retinas. Representative gels are also shown.
because only sst₁ is expressed by SRIF-containing amacrine cells.¹¹,³¹,³²

**Dynamic Changes of sst₂₄ Localization after Hypoxia**

The localization studies demonstrate for the first time that hypoxia induces a drastic reduction in sst₂₄-IR in retinal cells and processes. Although acute exposure to hypoxia was recently shown to cause changes in Müller cells and degeneration of neural cells in the retinas of neonatal rats,⁴¹ the persistence of the cells known to express sst₂₄, including amacrine and rod bipolar cells,⁴² excludes the possibility that in the OIR model the observed reduction of sst₂₄ is caused by hypoxic damage to the cells expressing the receptor.

We observed that in hypoxic retinas, endothelial cells displayed relatively abundant sst₂₄-IR, whereas in normoxic retinas, sst₂₄-IR was scarcely associated with retinal vessels. This result is in line with the finding that quiescent human vascular endothelial cells do not express sst₂ and this receptor is expressed when the endothelial cells begin to grow.⁴³,⁴⁴ In addition, our observations are also consistent with studies in human eyes with choroidal neovascularization in which newly formed endothelial cells strongly express sst₂.⁴⁵ Finally, the pattern of sst₂ expression in patients with diabetic retinopathy indicates that beneficial effects of sst₂ agonists may depend on the presence of sst₂ on newly formed vessels.⁴⁶ Our results on hypoxia-induced upregulation of sst₂₄ expression in retinal vessels suggest that sst₂₄ in the growing endothelium can receive angiinhibitory action of SRIF analogues with high affinity for sst₂. A relationship between sst₂ levels and octreotide efficacy has recently been demonstrated in rats with advanced stages of portal hypertension in which sst₂ becomes downregulated, and this downregulation can be responsible for the failure of octreotide therapy to inhibit angiogenesis.⁴⁷

**STAT3 as Downstream Effector**

Our recent work demonstrates that SRIF angiinhibitory effects initiated by sst₂ activation involve the downregulation of VEGF expression.⁴⁸ Consistent with such observations, octreotide has been found to be effective in reducing both choroidal neovascularization and VEGF mRNA levels in retinal pigment epithelium and choroidal tissue of rats.⁴⁹,⁵⁰ Therefore, it would be important to detect downstream effectors to trace a link between sst₂ activation and VEGF modulation. Much work has been done to clarify the functional role of transcription factors regulating target genes involved in angiogenesis.⁵¹-⁵⁴ Of these transcription factors, STAT3 appears of particular interest because its activation appears to be coupled to the regulation of VEGF.⁵⁵-⁵⁷,⁵⁸

STAT3 has been detected in the retina, though its role there has not been entirely determined.¹¹,³¹,³²,⁵⁹,⁶⁰ STAT3 expression and activation are increased in the OIR model,¹¹,¹²,¹₄ and activated STAT3 has been localized to retinal neovascular vessels.¹² In line with these results, we demonstrated that both STAT3 and pSTAT3 are upregulated by hypoxia in OIR mice suggesting that STAT3 may help to mediate the proliferation of blood vessels in the neovascular retina.

As also shown here, the upregulation of STAT3 in the hypoxic retina is influenced by treatment with the sst₂ agonist or antagonist. In fact, the hypoxia-induced increase of both STAT3 and pSTAT3 is consistently reduced by sst₂ activation with octreotide, indicating that ameliorative effects of octreotide on angiogenic responses in the hypoxic retina are likely to involve STAT3 production. In the OIR model, the beneficial effects of statins on retinal neovascularization have been reported to be associated with statin effects in preventing pSTAT3 upregulation.¹¹ In addition, in rats treated with streptozotocin to induce diabetes and in retinal endothelial cells maintained in high-glucose medium, the ameliorative effects of simvastatin seem to prevent STAT3 activation.⁵⁶

Our demonstration that octreotide prevents the upregulation of pSTAT3 associated with neovascularization in the OIR model is in line with previous results showing an inhibitory effect of octreotide on STAT3 activity in myeloid cells.⁵⁷ This inhibitory effect includes the activation of Src homology region 2 domain-containing phosphatase 1 (Shp-1), which in turn inhibits STAT3 activation. Interestingly, Shp-1 has been reported to be associated with the antiproliferative effect of sst₂ in tumor cells (see Ref. ⁵⁸ for review). Our additional finding that the hypoxia-induced increase in pSTAT3 is further enhanced by sst₂ blockade with CYN adds further evidence supporting sst₂ coupling to STAT3 in the mouse retina. Although there is indication of possible STAT3 location downstream of VEGF,¹¹,¹₂ we favor the hypothesis that the sst₂-induced inhibition of angiogenesis occurs through sst₂ functional coupling to STAT3 which, in turn, causes VEGF downregulation. The possibility that STAT3 activation is an upstream event with respect to hypoxia-induced VEGF upregulation is also suggested by results in bovine microvascular endothelial cells in which the activation of STAT3 is required to mediate the effects of peroxynitrite in stimulating VEGF expression.¹⁵ Additional investigations are necessary to verify the role of STAT3 in mediating the inhibitory effects of sst₂ activation on retinal angiogenesis and VEGF expression. Defining the pathway by which STAT3 mediates sst₂ action in retinal angiogenesis may provide new targets for the pharmacologic modulation of angiogenesis.

**Conclusions**

SRIF analogues with high specificity for sst₂ exert antiangiogenic effects by interfering with the VEGF system in the OIR model. Here, we demonstrate that low oxygen availability affects the levels and localization of sst₂ in the mouse retina and that this effect may be related to altered levels of SRIF. We also demonstrate that hypoxia influences the expression and activity of STAT3 and that the effects of hypoxia on STAT3 are reduced by sst₂ activation with octreotide. We suggest the possibility that sst₂ expressed by retinal capillaries exerts its angiinhibitory action through STAT3-induced modulation of VEGF. The present results further support the possibility of the use of sst₂-selective ligands in the treatment of retinopathy. Considering the eminent function of STAT3 in retinopathy, its targeting may represent a novel strategy for therapeutic intervention. However, it is important to underscore that although the OIR model used in the present study manifests the symptoms of retinal angiogenesis, it is not a model of proliferative diabetic retinopathy because the angiogenesis associated with proliferative diabetic retinopathy may well involve different mechanism(s) in diabetes.

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**References**

Expression and Functional Coupling of sst2

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