Antiangiogenic Role of Somatostatin Receptor 2 in a Model of Hypoxia-Induced Neovascularization in the Retina: Results from Transgenic Mice

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Purpose. To determine whether the somatostatin receptor 2 (sst_2) influences angiogenesis and its associated factors in a model of hypoxia-induced retinal neovascularization.

Methods. sst_1-knockout (KO) mice, in which sst_2 is overexpressed and overfunctional, and sst_2-KO mice were used. Angiogenesis was evaluated in fluorescein-perfused retinas. Angiogenesis-associated factors were determined by RT-PCR and immunohistochemistry.

Results. Retinal neovascularization was increased in sst_2-KO mice, but remained unchanged in sst_1-KO compared with wild-type (WT) mice. Retinal levels of sst_2 mRNA were not affected by hypoxia. Normoxic levels of angiogenesis regulators were similar in WT and KO retinas except for mRNA levels of IGF-1, Ang-2, and its receptor Tie-2. In WT, hypoxia induced an increase in mRNA levels of (1) VEGF and its receptors, (2) IGF-1R, and (3) Ang-2 and Tie-2. The increase in VEGF and IGF-1R mRNAs was more pronounced after sst_2 loss, but was less pronounced when sst_2 was overexpressed. In addition, in hypoxic retinas, sst_2 loss increased IGF-1 mRNA, whereas it decreased Ang-1, Tie-1, and Tie-2 mRNA levels. Moreover, Tie-1 mRNA increased when sst_2 was overexpressed. Immunohistochemistry confirmed the results in hypoxic retinas on increased expression of VEGF, IGF-1, and their receptors after sst_2 loss. It also allowed the localization of these factors to specific retinal cells. In this respect, VEGFR-2, IGF-1, and IGF-1R were localized to Müller cells.

Conclusions. These results suggest that sst_2 may be protective against angiogenesis. The immediate clinical importance lies in the establishment of a potential pharmacological target based on sst_2 pharmacology. (*Invest Ophthalmol Vis Sci.* 2007;48:3480–3489) DOI:10.1167/iovs.06-1469

The abnormal formation of new blood vessels characterizes a variety of retinal diseases, including diabetic retinopathy,[1] and requires the involvement of vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, and their receptors VEGFR-1, VEGFR-2, and IGF-1R. Although the available information on their expression in the retina is far from being exhaustive, these factors have been localized to both retinal cells and microvascular endothelium.[2–6] Retinal neoangiogenesis is always associated with an increase of VEGF and its receptors[2,3,7] but not of IGF-1.[8–10] Of the other downstream factors affecting blood vessel growth, recent results indicate that Ang-1 and -2 and their tyrosine kinase receptor Tie-2 are regulated by hypoxia and play a role in retinal neovascularization.[11,12] Although a ligand for Tie-1 has not been found, it has recently been demonstrated that Ang-1 can induce Tie-1 phosphorylation.[13]

The potential antiangiogenic role of the peptide somatostatin-14 (SRIF) and its analogues has received much attention,[14] and it involves partial correction of systemic growth hormone dysregulation or inhibition of angiogenesis-associated factors.[15–17] Of the five SRIF receptors mediating SRIF actions, sst_2 is a likely candidate to mediate the angioinhibitory activity of SRIF. Indeed, analogues with high affinity for sst_2, such as octreotide and BIM23027, counteract the growth factor–induced proliferation of bovine retinal endothelial cells under hypoxia.[18] They are powerful inhibitors of neovascularization in models of proliferative retinopathies.[15,19] In addition, octreotide inhibits the IGF-1-mediated induction of VEGF in human retinal pigment epithelial (RPE) cells.[16] Moreover, octreotide retards retinopathy progression in diabetic patients in whom photocoagulation has failed.[20] However, despite the growing use of sst_2 agonists as antiangiogenic agents, the mechanisms by which these peptides inhibit the growth of new vessels has not been fully delineated.

Both sst_1- and sst_2-knockout (KO) mice have been generated.[21,22] In their retinas, we have found that sst_1 loss causes an increased expression and function of sst_2.[23–28]

In the present study, retinas of sst_1- and sst_2-KO mice were rendered hypoxic and were used to investigate whether altered levels of sst_2 play a role in regulating retinal angiogenesis and its associated factors. Our hypothesis was that, compared with wild-type (WT) retinas, the lack of sst_2 is associated with heavier effects of hypoxia, whereas a chronic overexpression of sst_2 (as in sst_1-KO retinas) should attenuate these effects.

Methods

Animals

Experiments were performed on 128 mice of WT (C57BL/6) and sst_1- or sst_2-KO strains of both sexes at postnatal day (PD)17 (6 g body weight). In some experiments designed to evaluate development of retinal vasculature, five PD12 mice for each strain were also used. sst_1- and sst_2-KO mice were generated as previously reported.[21,22] 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 116/1992 and EEC/609/86. All efforts were made to reduce the number of animals used.

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