Deficit of Somatostatin-Like Immunoreactivity in the Vitreous Fluid of Diabetic Patients

Possible role in the development of proliferative diabetic retinopathy

Rafael Simó, MD1
Albert Lecube, PhD1
Laura Sararols, PhD2
José García-Arumí, MD2
Rosa M. Segura, MD3
Roser Casamitjana, MD4
Cristina Hernández, MD4

OBJECTIVE — To evaluate the vitreous levels of somatostatin-like immunoreactivity (SLI) in patients with proliferative diabetic retinopathy (PDR).

RESEARCH DESIGN AND METHODS — A total of 14 diabetic patients with PDR, in whom a vitrectomy was performed, were included in the study. Sixteen nondiabetic patients, with other conditions requiring vitrectomy, served as a control group. Both venous blood and vitreous samples were collected at the time of vitreoretinal surgery. Patients in whom intravitreous hemoglobin was detectable were excluded. In addition, a correction for plasma levels of SLI and intravitreal proteins was performed. SLI was measured by radioimmunoassay and vitreous hemoglobin by spectrophotometry.

RESULTS — SLI in the vitreous fluid was significantly lower in diabetic patients than in the control group (68 ± 18.7 vs. 193.6 ± 30.8 pg/ml, P < 0.01). The vitreous SLI-to-plasma SLI ratio was strikingly higher in nondiabetic control subjects than in diabetic patients with PDR (5.3 [1.2–71.1] vs. 0.6 [0.03–4.1], P < 0.01). After correcting for total vitreous protein concentration, SLI (pg/mg of proteins) remained significantly higher in nondiabetic control subjects than in diabetic patients with PDR (186 [51–463] vs. 7.5 [0.8–82], P < 0.0001). Remarkably, intravitreous levels of SLI were higher than those obtained in plasma in nondiabetic control subjects (193.6 ± 30.8 vs. 43.5 ± 10.7 pg/ml, P < 0.0001). Finally, a lack of relationship between plasma and vitreous levels of SLI was observed in both diabetic patients with PDR and nondiabetic control subjects.

CONCLUSIONS — The significantly higher SLI in the vitreous fluid than in plasma detected in nondiabetic control subjects supports the concept that somatostatin plays a relevant role in retinal homeostasis. In addition, the intravitreous deficit of SLI observed in diabetic patients with PDR suggests that it might contribute to the process of retinal neovascularization.

Diabetes Care 25:2282–2286, 2002

Somatostatin is a peptide that was originally identified as the hypothalamic factor responsible for inhibition of the release of growth hormone (GH) from the anterior pituitary (1). Subsequent studies have shown that somatostatin has a much broader spectrum of inhibitory actions and that it is much more widely distributed in the body, occurring not only in many regions of the central nervous system but also in many tissues of the digestive tract, including the stomach, intestine, and pancreas (2). Somatostatin-14 and -28 are the two principal bioactive products cleaved from the COOH-terminus of prosomatostatin in different cells and the main circulating forms of somatostatin (2,3). Somatostatin-13, converted from somatostatin-14 by the action of tissue aminopeptidases, is also present in plasma as prosomatostatin, and collectively all of these peptides contribute to the measurement of somatostatin-like immunoreactivity (SLI) (3). Somatostatin mediates its multiple biologic effects via specific plasma membrane receptors that belong to the family of G-protein–coupled receptors having seven transmembrane domains. So far, five somatostatin receptor subtypes (SSTRs) have been identified (SSTRs 1–5) (4).

The role of somatostatin in eye disease recently became of interest because of its role in proliferative diabetic retinopathy (PDR) and cystoid macular edema (5–10). Although the efficacy of somatostatin analogs in the treatment of advanced diabetic retinopathy has been largely attributed to their effectiveness in lowering serum IGF-I, they may also have an important direct antiproliferative effect on human retinal endothelial cells (11,12). In this regard, it must be pointed out that somatostatin is produced by the neuroretina of various species, including humans (13–18). Furthermore, SSTRs are also expressed in the retina, with SSTR1 and -2 being the most widely expressed (15,18–21). The production of both somatostatin and its receptors simultaneously suggests an autocrine action in the human retina (18–20).

Stimulation of SSTRs blocks angiogenesis in several model systems (22–24) and may also have a direct antiproliferative effect on human retinal endothelial cells.
During vitrectomy, partial posterior vitreous detachment was performed. For visualization of the vitreous cavity, we used a wide-field system with a precorneal Volk lens of 130° and inversion image system Moeller-Wedel (Hamburg, Germany). During vitrectomy, partial posterior vitreous detachment (PVD) was observed in 11 of 14 diabetic patients and in almost all nondiabetic subjects of the control group (6 of 8 macular holes and in all cases with either rhegmatogenous retinal detachment or epiretinal membranes).

Undiluted vitreous samples (0.5–1 ml) were obtained at the onset of vitrectomy by aspiration into a 1-ml syringe attached to the vitreous cutter (Ten-Thousand Outcome; Alcon, Irvine, CA) before starting intravitreal infusion of balanced salt solution. The vitreous samples were transferred to a sterile tube containing EDTA (0.054 ml, 0.34 mol/l) and aprotinin (500 KIU/ml), placed immediately on ice, and centrifuged at 16,000g for 5 min at 4°C. Supernatants were frozen at −80°C until assayed.

During vitrectomy, blood samples were collected in chilled tubes containing EDTA (0.054 ml, 0.34 mol/l) and aprotinin (500 KIU/ml) and then centrifuged immediately at 3,000g for 10 min at 4°C. The plasma obtained was aliquoted and stored at −80°C until assayed. The protocol for sample collection was approved by the hospital ethics committee, and informed consent was obtained from patients.

**Laboratory assays**

**SLI measurement.** SLI was measured by radioimmunoassay (Euro-Diagnostica, Malmo, Sweden). Before assay, somatostatin was extracted using Vycor, a leached silica glass, as previously described (26). The extraction recovery was similar for plasma and vitreous samples (75–85%). 125I-thyrosine somatostatin was used as a tracer together with a highly specific rabbit antisomatostatin serum (initial dilution 1:30,000). The antigenic site is directed toward the central part of the molecule containing the tryptophan residue. The antiserum shows no cross-reaction with a wide range of hypothalamic, pancreatic, gastrointestinal, or pituitary hormones. This antibody cross-reacts with cysolic somatostatin (100%), Tyr1-somatostatin (100%), linear somatostatin (50%), and des-ala-gly-somatostatin (25%). The lower detection limit was 10 pg/ml. The intra-assay coefficient of variation was 8.3% for values ~27 pg/ml and 2.8% for concentrations ~94 pg/ml. All samples were diluted in parallel to the cyclic somatostatin standard. Validation of the assay has previously been reported in detail (26).

**Protein assay.** Vitreal proteins were measured by a previously validated microturbidimetric method with an autoanalyzer (Hitachi 917; Boehringer, Mannheim, Germany). This method, based on the benzetonium chloride reaction, is a highly specific method for the detection of proteins and has a higher sensitivity and reproducibility than the classic method of Lowry. The lowest level of proteins detected was 0.02 mg/ml. Intra- and interassay coefficients of variation were 2.9 and 3.7%, respectively.

**Vitreous hemoglobin.** Apart from excluding patients with recent vitreous hemorrhage, we also excluded those in which intravitreous hemoglobin was detected. For this purpose, vitreous hemoglobin levels were measured by spectrophotometry (Uvikon 860; Kontron Instruments, Zürich, Switzerland) using the classic method of Harboe for measuring plasma hemoglobin in micromolar concentration (27). This method has been further validated (28), and in our studies, the lowest limit of detection was 0.03 mg/ml.

**Statistical analysis**

Values of SLI were compared using Student’s t test. However, because of their skewed distribution, the statistical comparisons of the ratios (vitreous SLI/plasma SLI and vitreous SLI/intravitreal proteins) were performed by means of a nonparametric test (Mann-Whitney U test). The Spearman rank correlation coefficient was used to examine correlations. Levels of statistical significance were set at \( P < 0.05 \). All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS-PC). The results are expressed either as the means ± SE or median and range.

**RESULTS** — The main clinical characteristics of patients with PDR and nondiabetic control subjects are summarized in Table 1. SLI in the vitreous fluid was significantly lower in diabetic patients than in the control group (68 ± 18.7 vs. 193.6 ± 30.8 pg/ml, \( P < 0.01 \)). By contrast, plasma SLI was higher in diabetic patients than in nondiabetic control subjects (88.2 ± 11.5 vs. 43.5 ± 10.7 pg/ml, \( P < 0.01 \)) (Fig. 1.).

We did not observe significant differences in SLI levels between type 1 and type 2 diabetic patients in either plasma (75.7 ± 18 vs. 100.6 ± 17 pg/ml, \( P = 0.34 \)) or the vitreous fluid (96.5 ± 24 vs. 52.6 ± 28 pg/ml, \( P = 0.25 \)). In addition, diabetic patients who received major laser treatment (\( n = 9 \)) presented similar SLI levels.
intravitreous concentration to those in whom retinal photocoagulation was not performed (n = 5; 62.55 ± 22.50 vs. 77.90 ± 56.51 pg/ml, P = 0.71).

Because vitreous SLI levels may in part reflect those in the plasma, we calculated the ratio of vitreous to plasma SLI concentrations in each patient. The vitreous SLI-to-plasma SLI ratio was strikingly higher in nondiabetic subjects than in diabetic patients with PDR (P < 0.01) (Fig. 2). This result was similar when the control group was compared separately with either type 1 (5.3 [1.2–71.1] vs. 1.0 [0.5–4.1], P < 0.01) or type 2 diabetic patients (5.3 [1.2–71.1] vs. 0.14 [0.03–1.94], P < 0.001).

We detected higher intravitreous protein concentrations in diabetic patients with PDR than in the control group (2.6 mg/ml [1.1–14.5] vs. 0.9 mg/ml [0.4–2.3], P < 0.0001). Therefore, after correcting for total vitreous protein concentration (vitreal SLI [pg/ml]–to–vitreal proteins [mg/ml] ratio), SLI (pg/mg of proteins) remained significantly higher in nondiabetic control subjects than in diabetic patients with PDR (186 [51–463] vs. 7.5 [0.8–82], P < 0.0001).

Remarkably, intravitreous levels of SST were significantly higher than those obtained in plasma in nondiabetic control subjects (193.6 ± 30.8 vs. 43.5 ± 10.7 pg/ml, P < 0.0001). By contrast, in diabetic patients, we observed higher levels of SLI in plasma than in the vitreous fluid, but in this case, the differences did not reach statistical significance (88.2 ± 11.5 vs. 68 ± 18.7 pg/ml, P = 0.59).

Finally, a lack of relationship between plasma and vitreous levels of SST was observed in both diabetic patients with PDR (r = 0.18, P = 0.55) and nondiabetic control subjects (r = −0.09, P = 0.72).

**CONCLUSIONS** — Vitreous fluid obtained from diabetic patients undergoing vitreoretinal surgery is currently used for indirectly exploring the synthesis of several peptides by the retina. However, we are unaware of previous reports in which SLI was determined in the vitreous fluid. In the present study, after considering the main confounding factors that could lead to misinterpretation of the results (vitreous hemorrhage, intravitreal protein concentration, and plasma SLI levels), we have provided evidence that there is a deficit of somatostatin in the vitreous fluid of diabetic patients with PDR. This finding was observed in absolute terms and was even overstated when either plasma levels of SLI or intravitreal protein concentrations were considered. It could be speculated that the high prevalence of PVD among diabetic patients with PDR could prevent the passage of somatostatin within the vitreous. However, the similar prevalence of PVD detected in both diabetic patients and nondiabetic control subjects makes its potential influence on the results highly unlikely. Another potential cause of the deficit of SLI found in the vitreous fluid of diabetic patients could have been previous photocoagulation. In this regard, it should be emphasized that we excluded all the patients who had received laser treatment in the preceding 3 months. In addition, we did not find any significant difference in vitreous SLI concentration between diabetic patients receiving laser treatment and those in whom retinal photocoagulation was not performed. Therefore, the influence of previous photocoagulation, at least in the group of patients included in the present study, would appear to be negligible.

Remarkably, higher SLI concentrations in the vitreous fluid than in plasma were detected in nondiabetic control subjects. It must be emphasized that the intravitreous protein concentration is at least 20-fold less than in serum (29,30). Thus, the higher intravitreal concentration of a particular protein in relation to its plasma levels strongly supports its intraocular production. Moreover, SLI levels found in the vitreous fluid of nondiabetic subjects included in our study were even higher than those observed by several authors in the cerebrospinal fluid of normal healthy control subjects (31–33). These findings suggest that soma-
somatostatin exerts an important role in retinal homeostasis. In this regard, there is growing evidence that in the retina, somatostatin acts as a neuromodulator through multiple pathways, including intracellular Ca²⁺ signaling (34), nitric oxide function (35), and glutamate release from photoreceptors (36). In addition, the loss of somatostatin immunoreactivity was found after degeneration of the ganglion cells (37). Therefore, the neuroretinal damage that occurs in diabetic retinopathy might be the reason for the decreased SLI detected in the vitreous fluid of these patients. Similarly, levels of SLI have been consistently decreased in the cerebrospinal fluid of patients with various neurodegenerative diseases (33, 38–41).

The observation that GH secretion is reduced by somatostatin analogs has been the basis for the clinical trials in severe PDR (5–7, 9, 10). Animal models also support the role of somatostatin analogs in the suppression of GH and IGF-I production and the subsequent inhibition of retinal neovascularization (25, 42). However, somatostatin may reduce endothelial cell proliferation and neovascularization by multiple mechanisms, including inhibition of postreceptor signaling events of peptide growth factors such as IGF-I, vascular endothelial growth factor, epidermal growth factor, and platelet-derived growth factor (11). In addition, both SSTR2- and SSTR3-selective analogs directly inhibit retinal endothelial cell growth in vitro (12, 43). Therefore, it appears that the inhibitory effect of somatostatin on retinal endothelial cell proliferation can be achieved independently of the modulation of systemic GH and IGF-I levels. In recent years, several natural inhibitors of angiogenesis that counterbalance the inducers of angiogenesis have been identified. The major natural inhibitor in the vitreous and cornea of the eye is pigment epithelium-derived factor (PEDF), a protein produced by retinal pigment epithelial cells and found in high concentrations within the retina as well as in the vitreous, where it is responsible for the antiangiogenic activity of this fluid (44). Furthermore, a deficit of PEDF has been found in the vitreous fluid of patients with PDR (45, 46), thus suggesting that the loss of angiogenic inhibitors has a central role in mediating the angiogenic response of retinal ischemia, such as that seen in PDR. The strikingly higher SLI concentration observed in nondiabetic control subjects lead us to propose somatostatin as a good candidate to be added to these natural inhibitors of angiogenesis. In addition, it is possible that the decreased concentration of SLI observed in the vitreous fluid of diabetic patients might contribute to the process of retinal neovascularization. However, because SLI vitreous concentration does not necessarily imply somatostatin activity, future studies exploring the binding to SSTRs are needed.

In summary, we have detected significantly higher SLI in the vitreous fluid than in the plasma in nondiabetic control subjects. This raises the possibility that SLI could be relevant for retinal homeostasis. In addition, we have observed a significant deficit of SLI in the vitreous fluid of diabetic patients. Obviously, further studies are needed to establish the cause of this finding as well as the potential role in the etiopathogenesis of diabetic retinopathy.

Acknowledgments—This work was supported by grants from the Ministerio de Sanidad y Consumo (FIS 98/1270), the Ministerio de Ciencia y Tecnología (PM 99-0136), and Novo Nordisk Pharma SA (01/0066). We thank Dr. F. Campos for the analysis of vitreous hemoglobin, Dr. Magela Garat and Dr. Ana Cantón for their contribution to the vitreous collection and processing of samples, and Michael Willy for his assistance with manuscript preparation.

References


12. Wilson SH, Davis MI, Caballero S, Grant...
Somatostatin and diabetic retinopathy

17. Sagar SM, Marshall PE: Somatostatin-like
15. Johnson J, Rickman DW, Brecha NC: So-
16. Rorstad OP, Senterman MK, Hoyte KM, 
20. Helboe L, Moller M: Immunohistochem-
21. Klisovic DD, O
22. Lawnicka H, Stepien H, Wyczolkowska J, 
24. Danesi R, Agen C, Benelli U, Paolo AD, 
A, Taccia MD: Inhibition of experimental
angio genesis by somatostatin analogue
octeotide acetate (SMs 201–995). Clin
25. Smith LEH, Kopchick JJ, Chen W, Knapp 
J, Kinose F, Daley D, Foley E, Smith RG, 
Schaeffer JM: Essential role of growth hor-
mone in ischemia-induced retinal neo-
vascularization. Science 276:1708–1709, 
1997
Stewart J, Dawson AM, Besser GM, Rees LH: Development and validation of a spe-
cific radioimmunoassay for somatostatin 
in human plasma. Ann Clin Biochem 16: 
15–23, 1979
27. Harboe M: A method for determination of 
hemoglobin in plasma by near-ultraviolet 
spectrophotometry. Scand J Clin Lab Invest 11: 
66–70, 1959
28. Fairbanks VF, Ziesmer SC, O’Brien: Meth-
ods for measuring plasma hemoglobin 
in micromolar concentration. Clin 
Chem 38:132–140, 1992
29. Burgos R, Mateo C, Cantón R, Hernán-
dez C, Mesa J, Simó R: Vitreous levels of 
IGF-1, IGF binding protein 1, and IGF 
binding protein 3 in proliferative diabetic 
retinopathy: a case-control study. Diabe-
tes Care 23:80–83, 2000
30. Hernández C, Burgos R, Cantón A, Gar-
cia-Arumí, Segura RM, Simó R: Vitreous 
levels of vascular adhesion molecule and 
vascular endothelial growth factor in pa-
tients with proliferative diabetic retino-
31. Cramer H, Heitzelmann KM: Somatosta-
tin in cerebrospinal fluid after generalized 
convulsions or cerebral infarction in hu-
mans. J Neurol Neurosurg Psychiatry 53: 
1015–1016, 1990
32. Maeda K, Yasuda M, Kaneda H, Maeda S, 
Yamadori A: Cerebrospinal fluid (CSF) 
levels of vascular adhesion molecule and 
vascular endothelial growth factor secretion from 
murine en-
33. Roca CA, Su TP, Elpern S, McFarland H, 
Pfeiffer AF: Loss of the antiangiogenic 
activity of somatostatin in vivo. Invest 
34. Akopian A, Johnson J, Gabriel R, Brecha 
N, Wikowsky P: Somatostatin modulates 
vascular-gated K (+) and Ca (2+) currents 
in rod and cone photoreceptors of the 
936, 2000
35. Smith LEH, Kopchick JJ, Chen W, Knapp 
J, Kinose F, Daley D, Foley E, Smith RG, 
Schaeffer JM: Essential role of growth hor-
mone in ischemia-induced retinal neo-
vascularization. Science 276:1708–1709, 
1997
36. Akopian A, Johnson J, Gabriel R, Brecha 
N, Wikowsky P: Somatostatin modulates 
vascular-gated K (+) and Ca (2+) currents 
in rod and cone photoreceptors of the 
936, 2000
37. Lake N, Patel YC: Neurotrophic agents re-
duce retinal somatostatin. Brain Res 181: 
234–236, 1980
38. Dupont E, Christensen SE, Hansen AP, 
Olivarius BD, Orskov H: Low cerebrospi-
nal fluid somatostatin in Parkinson’s 
disease: an irreversible abnormality. Neu-
rology 32:312–314, 1982
39. Vesci L, Cale B, Widerlov E, Elemann R, 
Czopf J, Pfaffly G: Lumbar cerebrospinal 
fluid concentrations of somatostatin and 
neuropeptide Y in multiple sclerosis. 
40. Edvinsson L, Minthon L, Elemann R, 
Gustafson L: Neuropeptides in cerebro-
spinal fluid of patients with Alzheimer’s 
disease and dementia with frontotempo-
ral lobe degeneration. Dementia 4:167– 
171, 1993
41. Molchan SE, Hill JL, Martinez RA, Lawlor 
BA, Mellow AM, Rubinow DR, Bissette G, 
Nemeroff CB, Sunderland T: CSF soma-
tostatin in Alzheimer’s disease and major 
depression: relationship to hypothalamic-
pituitary-adrenal axis and clinical mea-
sures. Psychoneuroendocrinology 18:509– 
519, 1993
42. Smith LE, Shen W, Perruzzi C, Soker 
K, Kinose F, Xu X, Robinson G, Driver 
S, Bischoff J, Zhang B, Schaeffer JM, 
Senger DR: Regulation of vascular endo-
thelial growth factor-dependent retinal neovascularization by insulin-like growth 
43. Grant MB, Caballero S, Millard WJ: Inhi-
bition of IGF-1 and bFGF stimulated growth of human retinal endothelial cells 
by the somatostatin analogue, octreotide, 
a potential treatment for ocular neovas-
44. Dawson DW, Volpen OV, Gillis P, Craw-
ford SE, Xu H-J, Benedict W, Bouck NP: 
Picatinysomatostatin-derived factor: a po-
tent inhibitor of angiogenesis. Science 
45. Ogata N, Tombran-Tink J, Nishikawa M, 
Nishimura T, Mitsuma Y, Sakamoto T, 
Matsumura M: Pigment epithelium-de-
rived factor in the vitreous is low in 
diabetic retinopathy and high in rhega-
matogenous retinal detachment. Am J Oph-
46. Spranger J, Osterhoff M, Reimann M, 
Mühlh M, Ristow M, Francis MK, Cristo-
falo V, Hennes HP, Smith G, Boulton M, 
Pfeiffer AF: Loss of the angiogenic 
pigment epithelium-derived factor in patients with angiogenic eye disease. Da-
abetes 50:2645–2649, 2001