

# Lower Somatostatin Expression Is an Early Event in Diabetic Retinopathy and Is Associated With Retinal Neurodegeneration

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**OBJECTIVE** — To test the hypothesis that a reduction of somatostatin (SST) in the retina exists in patients without clinically detectable diabetic retinopathy and that it is associated with retinal neurodegeneration.

**RESEARCH DESIGN AND METHODS** — Human diabetic postmortem eyes ( $n = 10$ ) without clinically detectable retinopathy were compared with eyes ( $n = 10$ ) from nondiabetic donors. SST mRNA levels (RT-PCR) and SST-28 immunoreactivity (confocal laser) were measured separately in neuroretina and retinal pigment epithelium (RPE). In addition, SST-28 (radioimmunoassay) was measured in the vitreous fluid. Glial fibrillar acidic protein (GFAP) was assessed by immunofluorescence and Western blot. Apoptotic cells were quantified using transferase-mediated dUTP nick-end labeling.

**RESULTS** — A higher expression of SST was detected in RPE than neuroretina in both groups. SST mRNA levels and SST-28 immunoreactivity were significantly lower in both RPE and the neuroretina from diabetic donors compared with nondiabetic donors. These results were in agreement with those obtained by measuring SST-28 in the vitreous fluid of the same donors. Increased GFAP and a higher degree of apoptosis were observed in diabetic retinas compared with nondiabetic retinas. These changes were most evident in patients with the higher deficit of SST.

**CONCLUSIONS** — Underproduction of SST is an early event in the eyes of diabetic patients and is associated with glial activation and neural death. In addition, our results suggest that RPE is an important source of SST in the human eye. The possible role of the lower production of SST in the pathogenesis of diabetic retinopathy requires further investigation.

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**D**iabetic retinopathy is the most common complication of diabetes and remains the leading cause of new blindness among working-age individuals in developed countries (1). Although

diabetic retinopathy has been traditionally viewed as a disorder of retinal vasculature, retinal neurodegeneration may be a primary pathology that gives rise to microvascular changes (2,3). The degenera-

tive changes in the neuroretina include increased apoptosis, glial cell reactivity, microglial activation, and altered glutamate metabolism. When occurring together, these changes may explain some of the functional deficits in vision appearing in diabetes before the onset of vascular abnormalities. In fact, abnormal electroretinograms have been found in patients with type 1 diabetes previous to the development of clinically detectable vascular retinal pathology (4), as well as in rats at short time intervals after the onset of experimental diabetes (5).

Somatostatin (SST) is a widely distributed peptide, and its diverse biological functions include neurotransmission and antisecretory and antiproliferative activities (6). SST and its receptors are found in the neuroretina of various species, including humans, and growing evidence suggests that in the retina, SST acts both as a neuromodulator and antiangiogenic factor (7). SST production in the human retina has been attributed to the neuroretina, predominantly in amacrine cells (8). However, whether retinal pigment epithelium (RPE) is also able to produce SST is unknown.

In recent years, we have demonstrated higher levels of SST in the vitreous fluid than in plasma in nondiabetic subjects, and a significantly lower intravitreal concentration of SST was detected in both proliferative diabetic retinopathy (PDR) (9) and diabetic macular edema (DME) patients (10) than in nondiabetic control subjects; SST-28 was the main molecular variant accounting for this deficit (11). Although vitreous fluid analysis can be used as a surrogate for events in the retina, studies to evaluate SST expression in the retina in diabetic patients are needed. Ocular globes from diabetic donors are unique entities that not only allow for confirmation that SST production is impaired in the diabetic retina but also allow for exploration regarding whether this event occurs in the early stages of diabetic retinopathy.

The aim of the present study was to compare, for the first time, mRNA SST

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**Abbreviations:** DME, diabetic macular edema; GFAP, glial fibrillar acidic protein; PDR, proliferative diabetic retinopathy; RPE, retinal pigment epithelium; SST, somatostatin; TUNEL, transferase-mediated dUTP nick-end labeling.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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and SST immunoreactivity in the human retina from diabetic and nondiabetic donors. Retinal apoptosis and glial activation were also investigated. Because SST is produced in the neuroretina and retinal neurodegeneration is an early event in diabetic retinopathy, we tested the hypothesis that a reduction of SST associated with retinal neurodegeneration exists in patients without clinically detectable diabetic retinopathy.

## RESEARCH DESIGN AND METHODS

**Ten human postmortem eyes** were obtained from five diabetic donors who were free of fundoscopic abnormalities according to ophthalmologic examinations performed during the previous 2 years. Ten eye cups obtained from five nondiabetic donors matched by age were used as the control group. The eye cups were obtained between March 2005 and January 2006. During this period, we obtained 10 eye cups from 5 consecutive diabetic donors and 42 from 21 nondiabetic donors. All diabetic donors were included in the study, whereas only five nondiabetic donors (10 eyes), closely matched by age, were selected. In the medical records of these donors, no concomitant diseases were found that could influence circulating SST levels. The main features of both groups are shown in Table 1 of the online appendix (available at <http://care.diabetesjournals.org>). The time elapsed from death to eye enucleation was  $3.8 \pm 1.1$  h, and no differences were observed between diabetic and nondiabetic donors ( $3.4 \pm 1.1$  vs.  $4.2 \pm 0.9$  h,  $P = \text{NS}$ ).

After enucleation, one eye of each donor was snap frozen at  $-80^{\circ}\text{C}$  and stored until assayed for mRNA and Western blot analyses. The other was fixed in 4% paraformaldehyde and embedded in paraffin for the immunohistochemical study.

All ocular tissues were used in accordance with applicable laws and with the Declaration of Helsinki for research involving human tissue and were approved by the ethics committee of our hospital.

## SST measurements

**mRNA isolation and cDNA synthesis.** Neuroretina and RPE were harvested under the microscopic dissection of isolated right eye cups from donors. Poly A<sup>+</sup> mRNA from tissue samples was isolated using Dynabeads oligo (dT)<sub>25</sub> (Invitrogen, Eugene, OR). mRNA concentration was determined by spectrophotometric measures at 260 and 280 nm. Aliquots of

poly A<sup>+</sup> mRNA (200 ng) were reverse transcribed using the Cloned AMV First-Strand cDNA synthesis kit (Invitrogen) following the manufacturer's protocol for oligo (dT)<sub>20</sub> priming. The resulting cDNA was then used in both semiquantitative and real-time PCRs.

**Semiquantitative RT-PCR.** SST mRNA was evaluated by semiquantitative RT-PCR using 2  $\mu\text{l}$  of the first-strand cDNA as template and gene-specific primers. Amplification of the cDNA samples with  $\beta$ -actin primers served as an endogenous control. In parallel with cDNA samples, the reactions were also performed without DNA template to exclude contamination of the PCR mixtures. cDNA samples known to contain SST mRNA were amplified as a positive control. To insure that poly A<sup>+</sup> mRNA was not contaminated with genomic DNA, cDNA reactions were also performed without reverse transcriptase and amplified with each primer pair. PCR was performed using Platinum TaqDNA polymerase (Invitrogen). Sequences for primers used in semiquantitative RT-PCR for SST and  $\beta$ -actin were designed using published gene sequences and Primer Select software (DNASar, Madison, WI). The sequences of the primer pairs were as follows: 1) SST (size 270 bp): forward, 5'-GTCCTGGCCCTGGCTGTGTAC-3'; reverse, 5'-TGCTGTCTCGGGGTGCCATAGC-3'. 2)  $\beta$ -Actin (size 495 bp): forward, 5'-ATCGTGCGTGACATTAAGGAGAAGC-3'; reverse, 5'-AGAAGCATTGCGGTGGACGAT-3'. The reaction mixture contained 5  $\mu\text{l}$  10 $\times$  PCR buffer (200 mmol/l Tris-HCl, pH 8.4, 500 mmol/l KCl), 1  $\mu\text{l}$  of 10 mmol/l dNTP, 1.5  $\mu\text{l}$  of 50 mmol/l MgCl<sub>2</sub>, 1  $\mu\text{l}$  of each 10  $\mu\text{mol/l}$  forward and reverse primers, 1 unit Platinum Taq polymerase, and 2  $\mu\text{l}$  of cDNA from RT reaction. Identities of the products were confirmed by direct sequencing using an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Madrid, Spain).

**Quantitative real-time RT-PCR.** Quantitative real-time RT-PCR was performed using an ABI Prism 7000 Sequence Detection System (PE Applied Biosystems), according to the manufacturer's protocol. The reactions were performed using TaqMan Universal PCR Master Mix (PE Applied Biosystems), 2  $\mu\text{l}$  cDNA template, and Assays-on-Demand Gene Expression Assay Mix (Hs00174949\_m1; PE Applied Biosystems), which includes specific probe and primers, in a total volume of 20  $\mu\text{l}$ . Each sample was assayed in triplicate,

and control negative samples were included in each experiment. Human  $\beta$ -actin was used as an endogenous gene expression control (4333762T; PE Applied Biosystems).

**SST-28 immunofluorescence.** For immunohistochemical study, SST-28 rather than SST-14 was selected because it is more abundant in the vitreous fluid and is the main molecular variant accounting for the deficit of intravitreal SST detected in diabetic patients (11).

Paraffinized eyes were serially cut at 7- $\mu\text{m}$  thickness. Sections were deparaffinized with xylene and rehydrated in ethanol. Sections were then fixed and placed in antigen-retrieval solution (Dako, Glostrup, Denmark) for 20 min at  $95^{\circ}\text{C}$ . To eliminate the autofluorescence of RPE due to melanin and lipofuscin, we used a method described elsewhere (12). Sections were then incubated for 1 h with 1% BSA in 0.3% Triton X-100 in PBS to block unspecific binding of the antibodies and then incubated overnight at  $4^{\circ}\text{C}$  with a specific primary antibody to human SST-28 (Abcam, Cambridge, U.K.). After washing, sections were incubated with Alexa Fluor 633 (Molecular Probes, Eugene, OR) secondary antibody at room temperature for 1 h. Slides were cover slipped with a drop of mounting medium containing 4',6-diamidino-2-phenylindole for visualization of cell nuclei (Vector Laboratories, Burlingame, CA).

Images were acquired with a confocal laser scanning microscope (FV1000; Olympus, Hamburg, Germany), using a 633-nm laser line for SST-28 and a 405-nm laser for 4',6-diamidino-2-phenylindole fluorophores. Each image was saved at a resolution of  $1,024 \times 1,024$  pixel image size.

To quantify SST-28 immunofluorescence in RPE and the neuroretina, the total fluorescence intensity values corresponding to 10 field frame images ( $40\times$  NA: 0.9) of each retina sample were measured. These results were then normalized, taking into account the area analyzed. All these calculations were made using specific software (Fluoview ASW 1.4). We also measured the thickness of the entire neuroretina corresponding to five fields frame images ( $40\times$ ).

**SST-28 in vitreous fluid.** SST-28 levels were measured by radioimmunoassay (Phoenix Pharmaceutical, Belmont, CA). The antibody used against SST-28 was highly specific and recognized 100% of SST-28 and did not have a cross-reaction with either SST-14 or cortistatin (11). The

lower detection limit was 20 pg/ml. Intravitreal proteins were determined by the Bradford method (13) using BSA as the standard.

### Transferase-mediated dUTP nick-end labeling

Paraffin-embedded eye sections (7- $\mu$ m thickness) from diabetic and nondiabetic donors were processed with an apoptosis detection kit (APO-BrdU TUNEL Assay kit; Molecular Probes). The methodology used is described in detail in the online appendix. Transferase-mediated dUTP nick-end labeling (TUNEL) staining in the diabetic retina was compared with that in the nondiabetic retina. For this purpose, each retina was visually scanned with a high-power lens (60 $\times$ ), which covers 212  $\times$  212. The total number of TUNEL-positive cells was recorded for each retina in a masked fashion using the values corresponding to 15 fields frame images from three retinal sections (five fields each). The mean value was then standardized (mathematically converted) to a 0.5-cm<sup>2</sup> area. To avoid false-positive results (13), we have only considered as apoptotic those TUNEL-positive cells in which nuclear fragmentation and/or condensation was present. For this purpose, we used interdigital contrast (phase-contrast microscopy) and propidium iodide immunofluorescence.

### Glial fibrillar acidic protein assessment

**Western blot.** Neuroretina samples were transferred into a lysis buffer (1% Triton X-100, 100 mmol/l Tris-HCl, pH 7.5; 150 mmol/l NaCl; 1.5 mmol/l EDTA, pH 8.0; 25 mmol/l NaF; 0.5 mmol/l Na<sub>3</sub>VO<sub>4</sub>; 0.1 mmol/l phenylmethylsulfonyl fluoride; and Complete protease inhibitors [Roche, Basel, Switzerland]) and then homogenized by sonication. The protein concentration was determined using Bradford assay (Bio-Rad Laboratories, Sundbyberg, Sweden). A total of 5  $\mu$ g protein from the neuroretina was resolved by 12% SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA). The membranes were incubated with a primary antibody against human glial fibrillar acidic protein (GFAP) (Abcam), diluted 1:200,000, and further incubated with peroxidase-conjugated secondary antibody (Bio-Rad Laboratories). Proteins were visualized using the enhanced chemiluminescence detection system (Supersignal CL-HRP Substrate System;

Pierce, Rockford, IL). The same blot was stripped and reblotted with an antibody specific to  $\beta$ -actin (Santa Cruz Biotechnology, Santa Cruz, CA) to normalize the GFAP levels. Densitometric analysis of the autoradiographs was performed with a GS-700 Imaging Densitometer (Bio-Rad Laboratories) and analyzed with Quantity One 4.6.2 software (Bio-Rad Laboratories).

**Immunofluorescence.** Tissue sections were incubated overnight at 4°C with the primary antibody chicken anti-human GFAP (1:1,000; abcam). After washing, sections were incubated with Alexa Fluor 488 (Molecular Probes) secondary antibody for 1 h. GFAP immunofluorescence in the neuroretina was quantified using a laser confocal scanning microscope. The procedure was the same as mentioned above for SST-28.

**Retinal thickness.** Retinal thickness was measured in each retina from the apical edge of outer nuclear layer to the internal limit of ganglion cell layer. Five fields frame image (40 $\times$  confocal microscopy) were used for each retina and the mean value calculated.

### Statistical analysis

Results are expressed as means  $\pm$  SD. The comparisons between groups (diabetic and nondiabetic donors) or between tissues (RPE and neuroretina) were performed using the Student's *t* test. Correlation analyses were performed using Spearman's correlation coefficient. *P* < 0.05 was considered statistically significant.

## RESULTS

### Expression of SST in the human retina

$\beta$ -Actin mRNA expression was similar in both the RPE and the neuroretina (*P* = NS). In addition, no differences were observed in  $\beta$ -actin mRNA expression between diabetic and nondiabetic retinas (*P* = NS). Thus, we have calculated SST mRNA gene expression after normalizing with  $\beta$ -actin.

The comparative SST mRNA expression of diabetic and nondiabetic retinas is shown in Fig. 1. SST mRNA expression was detected in the retinas from both nondiabetic and diabetic donors; it was higher in the RPE than in the neuroretina in all cases. SST mRNA levels obtained in the neuroretina and in the RPE from diabetic donors were lower than those obtained from nondiabetic donors. To

evaluate the quantitative expression of SST mRNA, a quantitative real-time RT-PCR was performed (Fig. 1). We detected a significantly higher expression of SST mRNA in the RPE than in the neuroretina in retinas from both nondiabetic donors ( $0.98 \pm 0.02$  vs.  $0.38 \pm 0.24$ , *P* < 0.001) and diabetic donors ( $0.44 \pm 0.30$  vs.  $0.11 \pm 0.09$ , *P* = 0.018). SST mRNA levels were lower in both the RPE and neuroretina from diabetic donors compared with nondiabetic donors ( $0.44 \pm 0.30$  vs.  $0.98 \pm 0.02$ , *P* = 0.001, and  $0.11 \pm 0.09$  vs.  $0.38 \pm 0.24$ , *P* = 0.018, respectively).

### SST-28 in the retina and vitreous fluid

Laser scanning confocal images of SST-28 immunofluorescence are displayed in Fig. 1. SST-28 fluorescence intensity was significantly higher in the RPE than the neuroretina in both nondiabetic and diabetic donors (Fig. 1). In addition, SST-28 was more abundant in both the RPE and neuroretina from nondiabetic donors compared with diabetic donors, but the differences were only significant for the neuroretina.

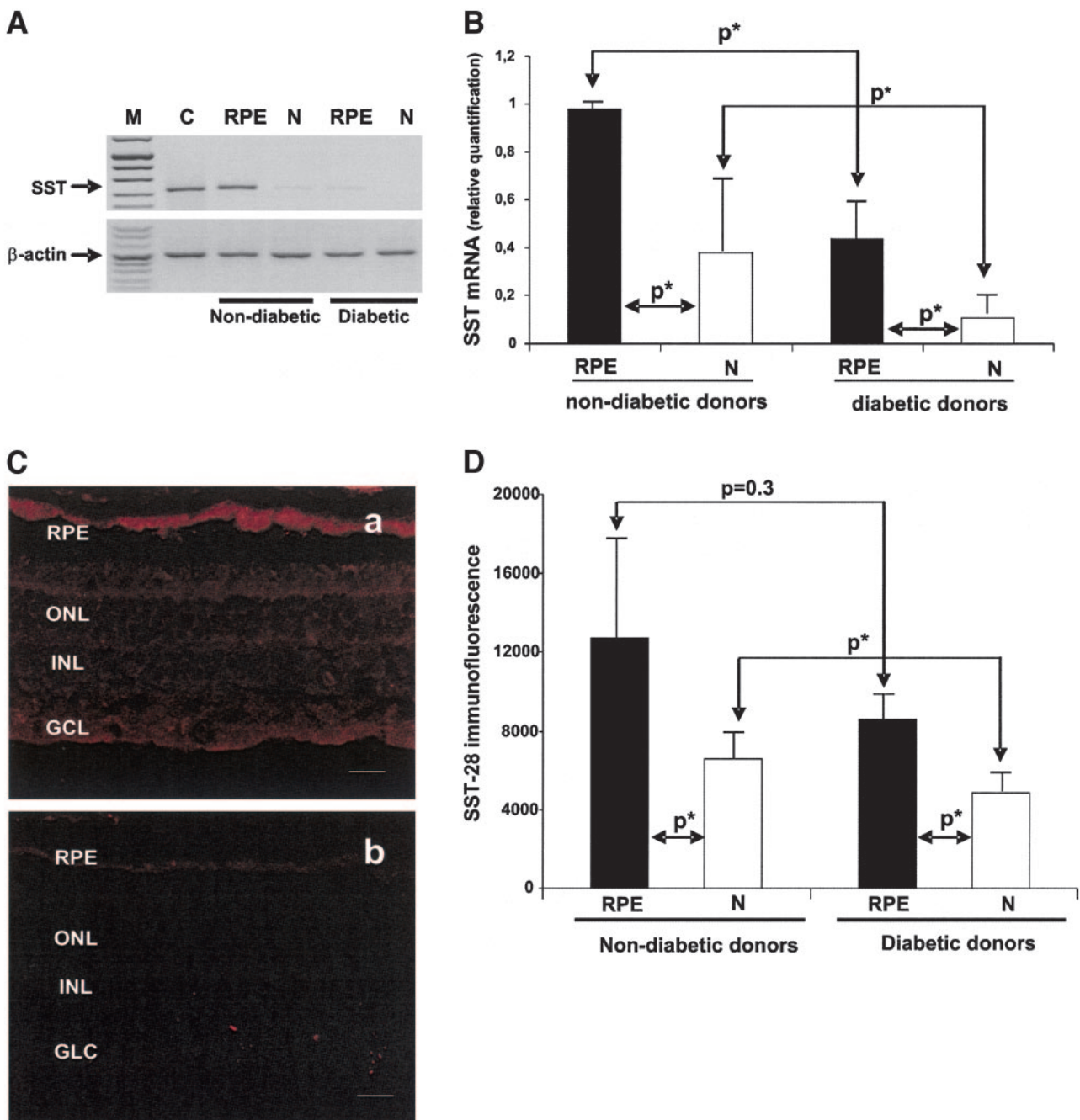
No differences in intravitreal protein levels between diabetic and nondiabetic donors were detected ( $1.24 \pm 0.42$  vs.  $1.06 \pm 0.28$  mg/ml, *P* = 0.40). Intravitreal levels of SST-28 were lower, but not statistically significant, in diabetic donors than in nondiabetic donors in absolute values ( $75 \pm 16$  vs.  $281 \pm 144$  pg/ml, *P* = 0.20) and after adjusting by intravitreal proteins ( $56.3 \pm 7.2$  vs.  $240 \pm 115$  pg/mg, *P* = 0.14).

### Effect of diabetes in retinal neurodegeneration: apoptosis and glial activation

Diabetic retinas had a significantly higher percentage of apoptotic cells than the age-matched nondiabetic retinas in both RPE ( $0.96 \pm 0.31$  vs.  $0.16 \pm 0.11\%$ , *P* < 0.0001) and neuroretina ( $0.36 \pm 0.14$  vs.  $0.07 \pm 0.06\%$ , *P* < 0.001). Furthermore, the number of apoptotic cells per cm<sup>2</sup> was higher in diabetic donors than nondiabetic donors in both RPE ( $184 \pm 78$  vs.  $42 \pm 28$ , *P* < 0.0001) and neuroretina ( $180 \pm 94$  vs.  $40 \pm 37$ , *P* < 0.0001).

In the neuroretinas, apoptosis was highest in the ganglion cell layer and lowest in the outer nuclear layer (Fig. 3 of the online appendix). This finding was observed in both diabetic and nondiabetic donors. The total thickness of the neuroretina was significantly lower in diabetic retinas compared with nondiabetic reti-





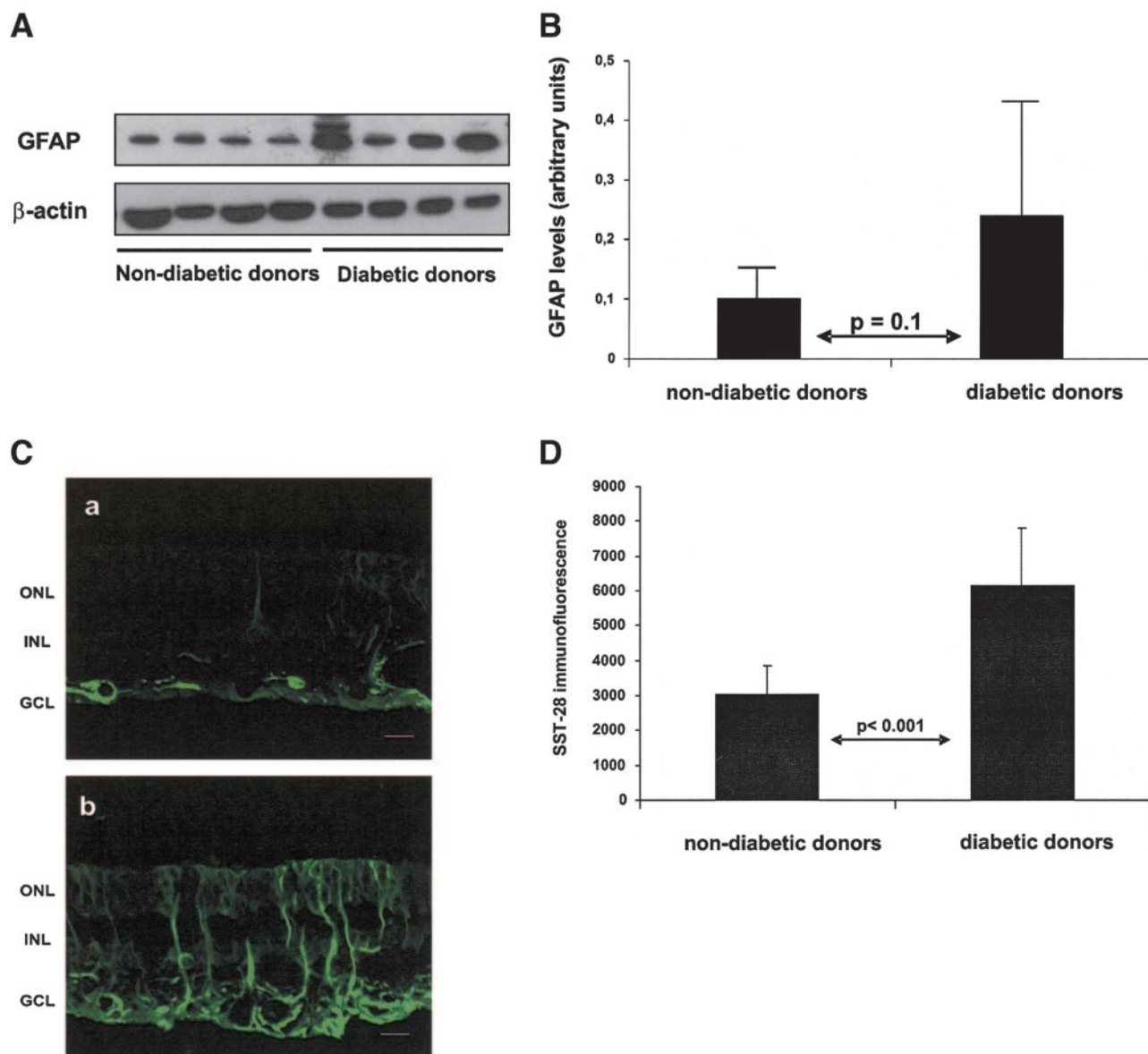
**Figure 1**—A: Expression of SST mRNA in the retina (RPE and neuroretina) of diabetic and nondiabetic donors. C, positive control (human brain); M, size marker; N, neuroretina. Human  $\beta$ -actin was used as internal control, and, as shown, its signal intensity was similar in the retina of diabetic and nondiabetic donors. B: Real-time quantitative RT-PCR analysis of SST mRNA in human retinas. Bars represent the means  $\pm$  SD of the values obtained in five diabetic and the five nondiabetic donors. SST mRNA gene expression was calculated after normalizing with  $\beta$ -actin. \* $P < 0.01$ . C: Comparison of SST-28 immunofluorescence (red) in the human retina between representative samples from a diabetic (a) and nondiabetic (b) donor. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. The bar represents 20  $\mu$ m. D: Quantification (relative fluorescence intensity levels) of SST-28 immunofluorescence in nondiabetic and diabetic retinas. \* $P < 0.01$ .

nas ( $103.8 \pm 26.1$  vs.  $127.1 \pm 26.2$   $\mu$ m,  $P = 0.001$ ).

A higher ratio of apoptosis was found in RPE than in neuroretina. This was true for both diabetic ( $0.96 \pm 0.31$  vs.  $0.36 \pm 0.14\%$ ,  $P = 0.01$ ) and nondiabetic ( $0.16 \pm 0.11$  vs.  $0.07 \pm 0.06\%$ ,  $P < 0.01$ ) retinas.

Neuroglial activation was demonstrated in diabetic eyes by an increase of GFAP production in the neuroretina. Densitometric analysis of immunoblots showed higher GFAP in the neuroretinas from diabetic donors compared with nondiabetic donors (Fig. 2). GFAP immunofluorescence was also significantly higher

in diabetic than in nondiabetic neuroretinas ( $6,131 \pm 1,857$  vs.  $3,038 \pm 1,590$ ,  $P < 0.001$ ) (Fig. 2). As previously described, Müller cells acquire prominent GFAP immunofluorescence throughout the extension of their processes in the diabetic retina, thus replacing the normal pattern of GFAP immunostaining (Fig. 2).



**Figure 2**—A: Western blot analysis of neuroretina corresponding to four representative diabetic and nondiabetic retinas. B: Densitometric quantification showing higher GFAP in the retinas from five diabetic donors compared with retinas from five nondiabetic donors ( $0.24 \pm 0.19$  vs.  $0.10 \pm 0.05$  arbitrary units/ $\mu$ g total protein,  $P = 0.16$ ). Results are expressed as means  $\pm$  SD. The relative intensities of the GFAP bands were normalized by those of the  $\beta$ -actin bands. C: Comparison of GFAP immunofluorescence (green) in the human retina between representative samples from a nondiabetic (a) and diabetic (b) donor. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. In the diabetic retina, the Müller cells' endfeet show abundant GFAP immunofluorescence and the radial processes stain intensely throughout both the inner and outer retina. The bar represents 20  $\mu$ m. D: Quantification (relative fluorescence intensity levels) of GFAP immunofluorescence in nondiabetic and diabetic retinas.

A significant inverse correlation was observed between SST-28 immunofluorescence and the percentage of apoptotic cells ( $r = -0.87$ ,  $P = 0.002$ ). Similarly, lower SST-28 immunoreactivity was detected in retinas with higher GFAP expression ( $r = -0.46$ ,  $P = \text{NS}$ ). Finally, a significant direct correlation was found between the percentage of apoptotic cells and GFAP immunofluorescence ( $r = 0.75$ ,  $P = 0.02$ ). Taken together, these findings strongly suggest that the higher the de-

gree of diabetic retinal neurodegeneration, the lower the rate of SST production.

**CONCLUSIONS**— The present study provides the first evidence that a significant reduction of both SST mRNA and SST-28 immunofluorescence exists in the diabetic retina and that it is associated with glial activation and neural death. It must be emphasized that, according to ophthalmologic examinations performed 2 years before death, the diabetic donors were

free of microvascular abnormalities, thus suggesting that a reduction of SST expression is an early event in the eyes of diabetic patients. In addition, it is worthy of note that we found RPE to be an important source of SST—both SST mRNA and SST-28 immunofluorescence were significantly higher in the RPE than in the neuroretina in both nondiabetic and diabetic donors.

The exact reason why diabetic patients have a lower SST mRNA expression

remains to be elucidated. The design of our study does not permit us to determine whether the lower expression of SST is a cause or a consequence of retinal apoptosis. However, it should be noted that SST has proapoptotic effects (14). Therefore, in the case that SST decrease was the primary mechanism, a lower rather than higher apoptosis would be expected in diabetic patients. In consequence, our results indicate retinal neurodegeneration and, in particular, retinal apoptosis as one of the mechanisms involved in the lower production of SST in diabetic patients. In fact, the higher the number of apoptotic cells, the lower the degree of SST-28 immunoreactivity. Nevertheless, given that SST has neuroprotective effects (15), the lower levels existing in diabetic patients could also contribute to retinal neurodegeneration.

Apoptotic cells have been observed in the neuroretina of diabetic experimental models (2,16,17) and in human diabetic neuroretinas (2,18). In the present study, neuronal apoptosis accounted for an 18% reduction in the thickness of the neuroretina, which is similar to that described by Barber et al. in rats after 7.5 months of diabetes (2). Apart from an elevated rate of apoptosis in diabetic neuroretinas, we found that apoptosis was even higher in RPE. Apoptosis of RPE is an important event in the pathogenesis of age-related macular degeneration, but it has not been previously reported in diabetic patients. Therefore, future studies should investigate the significance and potential role of apoptosis of RPE in the development of diabetic retinopathy.

As previously reported in experimental (19,20) and human (17,21) studies, we have detected an increased GFAP production by Müller cells in diabetic retinas. Experimental evidence shows that reactive changes in Müller cells, such as upregulation of GFAP, occur early in the course of the disease and precede the onset of overt vascular abnormalities (3). In the present study, we have confirmed this finding in human diabetic retinas. The mechanisms and significance of the increased GFAP content in diabetic retinas have not been well established. However, it has been reported that the upregulation of GFAP in Müller cells in the retinas of diabetic rats is inhibited by aldose reductase inhibitors (22,23) and by melatonin (24), suggesting that a hyperactive polyol pathway and oxidative stress contribute to the progression of Müller cell gliosis. In addition, Müller cell gliosis is character-

ized by responses to the inflammatory milieu present in the diabetic retina (25). It is worth mentioning that we have found a clear relationship between the number of apoptotic cells and GFAP immunoreactivity. This finding supports the concept that diabetes (and its related metabolic pathways) is a common mediator in these two processes.

The role of SST in retinal physiology and in the development of diabetic retinopathy is far from clear. Apart from its antiangiogenic properties, SST is a neuroactive peptide that influences retinal physiology (26). Recently, it has been demonstrated that SST modulates the nitric oxide/cGMP system, a signal transduction system that is of major importance in retinal physiology (27) and dopamine release (28). Furthermore, Yoshida et al. (29), in a microarray analysis of genes differently expressed in young and elderly human retinas, suggested that SST is essential in regulating retinal function. However, further studies are required to determine the precise relevance of SST deficit in the development of diabetic retinopathy.

Our findings may also have therapeutic consequences. Until now, the rationale for using SST analogs in the treatment of diabetic retinopathy has been based on their capacity to lower circulating IGF-1 levels. However, the role of serum IGF-1 in the development of diabetic retinopathy is controversial (30), and a direct neurotrophic and antiangiogenic effect in the retina rather than an indirect effect caused by a reduction in circulating IGF-1 possibly could be the main mechanism accounting for the effectiveness of SST analogs in diabetic retinopathy (12,31–34). Recently, we have shown lower levels of SST in DME (10). In the retina, various ion/water transport systems are located at the apical side of RPE adjacent to the subretinal space, and, indeed, a high expression of SST receptor 2 has been shown on this apical membrane of the RPE (8). Therefore, SST could modulate the balance of fluid and ion transport through the retina. In addition, SST and SST analogs have anti-inflammatory effects (35). Given that SST has antiedema and anti-inflammatory properties, it could be postulated that its deficit might be involved in the pathogenic events that lead to DME. Local delivery of SST analogs bypasses the blood-retinal barrier, allowing higher SST levels than could be achieved by systemic administration. The lower retinal expression of SST in diabetic do-

nors observed in the present study and our previous observation that intravitreal SST was lower in PDR and DME patients lead us to propose intravitreal delivery of SST as a replacement treatment for arresting or preventing both PDR and DME. Obviously, specific interventional studies using intravitreal injection of SST analogs or subretinal injections of viral vector encoding for SST gene therapy will be needed to confirm this hypothesis.

In conclusion, we provide evidence that SST is expressed not only in the neuroretina but also in the RPE. In addition, a reduction of SST expression and production is an early event in the diabetic retina and is associated with apoptosis and glial activation. Our findings suggest that a reduction in SST production may be involved in the development of diabetic retinopathy; if this is the case, new therapeutic strategies could be revealed.

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