Deficit of Somatostatin in the Vitreous Fluid of Patients With Diabetic Macular Edema

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Although diabetic macular edema (DME) is the main cause of visual loss in type 2 diabetic patients (1), its pathogenic mechanisms have been much less studied than proliferative diabetic retinopathy (PDR). The production of both somatostatin (SST) and its receptors by the retina suggests an autocrine action. However, the functional roles of this peptidergic system in retinal physiology are far from being fully elucidated. In recent years we have demonstrated that SST is decreased in the vitreous fluid from patients with PDR, SST-28 being the main molecular variant accounting for this deficit (2,3). Apart from angiostatic properties, SST has anti-inflammatory and antiedema effects (4–9). Therefore, it is possible that its deficit can be involved not only in PDR but also in DME development.

The aim of the present study was to determine whether, as occurs in PDR, a deficit of intravitreous SST also exists in DME, thus allowing us to identify a new potential pathogenic contributor to this devastating complication of diabetes. Since SST-28 is the main molecular variant in the vitreous fluid, it has been selected as the ideal candidate to be explored.

RESEARCH DESIGN AND METHODS

The study included 35 type 2 diabetic patients (15 with DME and 20 with PDR), in whom a classic three port pars plana vitrectomy was performed. Vitreous from 30 age-matched nondiabetic patients (18 with macular hole and 12 with idiopathic epiretinal membrane) served as the control group. The exclusion criteria were as follows: *1*) previous vitreoretinal surgery, *2*) recent vitreous hemorrhage or intravitreous hemoglobin >5 mg/ml, or *3*) photocoagulation in the preceding 3 months. Fluorescein angiography was performed in all patients with DME in order to assess the degree of retinal ischemia. Eyes with <10 disc areas of nonperfusion were classified as having little or no evidence of ischemia (10).

Vitreous and blood samples were collected simultaneously during vitrectomy and processed as previously described (3). SST-28 was measured by RIA (Phoenix Pharmaceuticals, Belmont, CA). The lower detection limit was 20 pg/ml. The antibody used in this RIA recognizes 100% of SST-28 and does not show cross-reactivity with either SST-14 or cortistatin (3). Vitreous hemoglobin levels and vitreal proteins were measured as previously described (3).

Statistical analysis

Student's *t* test and ANOVA were used to compare SST-28 concentrations, and the χ^2 test was used for categorical variables. Because of their skewed distribution, the statistical comparisons of intravitreal proteins were performed using a nonparametric test (Mann-Whitney *U* test). Correlations were examined by Spearman's rank correlation test. Levels of statistical significance were set at $P < 0.05$. Results are expressed as the mean ± SEM or median (range).

RESULTS

We did not observe significant differences in plasma SST-28 concentration among groups (DME 181 ± 28 pg/ml, PDR 189 ± 26 pg/ml, nondiabetic controls 136 ± 31 pg/ml; P = not significant). Fluorescein angiography demonstrated a lack of significant retinal ischemia in all patients with DME (mean of disc areas of retinal nonperfusion 0.9 [range 0–5]). Intravitreous protein concentration was significantly higher in diabetic patients than in control subjects (3.1 mg/ml [0.6–5.8] vs. 0.83 mg/ml [0.3–1.5], $P < 0.001$). However, no significant differences were detected between DME and PDR patients (2.9 mg/ml [1.4–5.8] vs. 3.2 mg/ml [0.6–5.1], $P = 0.74$).

The intravitreal SST-28 concentration was significantly lower in patients with DME than in control subjects in absolute terms and also after correcting for intravitreal proteins (Fig. 1). No difference in intravitreous SST-28 concentration was detected in subjects with DME in comparison with PDR patients in either absolute terms or after adjusting for intravitreal proteins. SST-28 concentration was 4.6-fold higher in vitreous fluid than in plasma from control subjects ($P < 0.0001$), whereas in diabetic patients the difference was only ~0.5-fold higher ($P = 0.02$). No correlation between intravitreal and plasma SST-28 concentration was detected in either diabetic patients or control subjects.

CONCLUSIONS

In the present study, we provide evidence that intravitreal SST-28 is markedly lower in patients with DME than in control subjects. Notably, the intravitreous deficit of SST-28 in DME was in the same range as that obtained in PDR. In addition, we have con-

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Abbreviations: DME, diabetic macular edema; PDR, proliferative diabetic retinopathy; SST, somatostatin.

The contribution of R.S. and E.C. should be considered equal.

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

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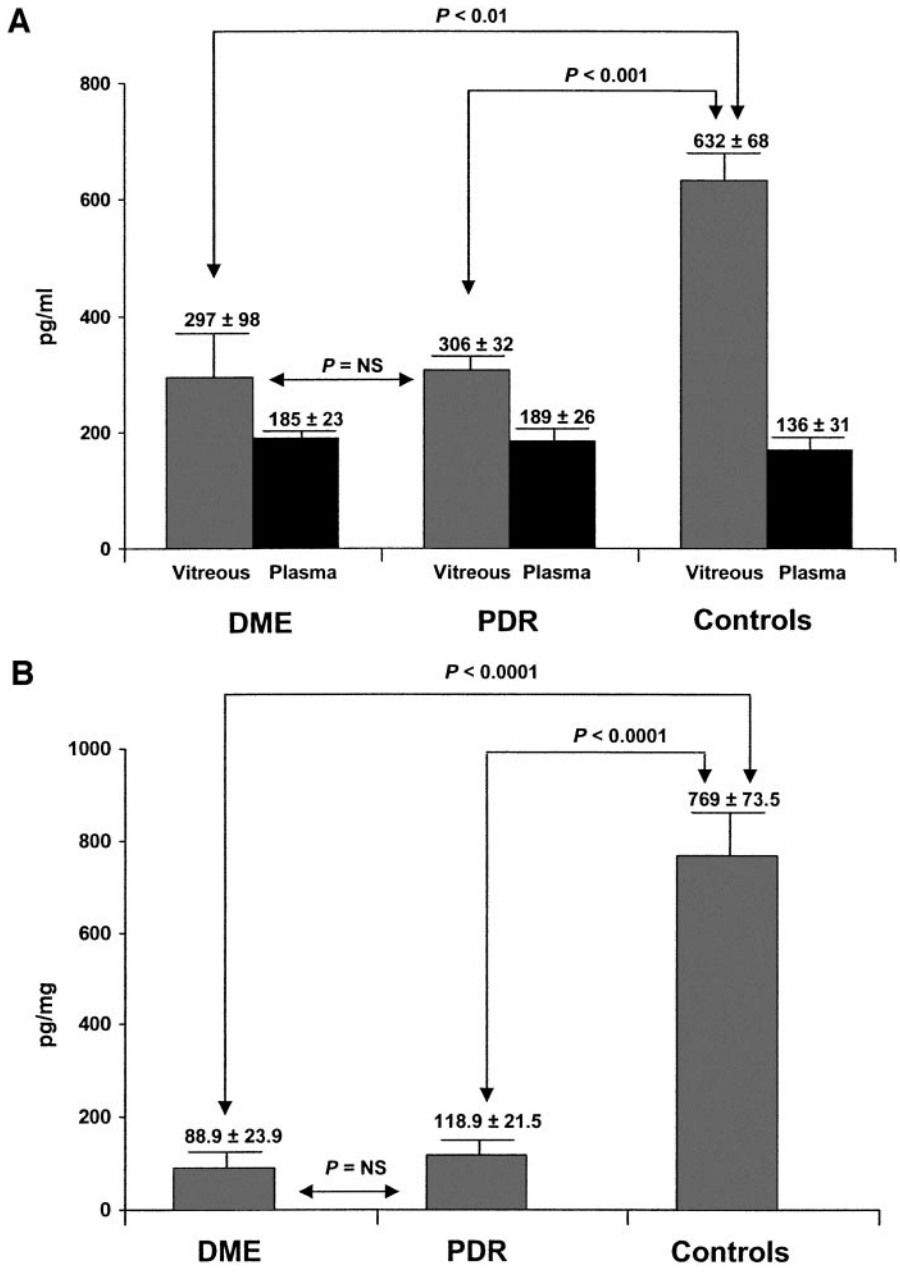

Figure 1— A*: Intravitreous and plasma concentrations of SST-28 (pg/ml) in patients with DME or PDR and in nondiabetic control subjects.* B*: Ratio of intravitreal SST-28 concentration to total intravitreal proteins (pg/mg). Values are expressed as the mean ± SEM.*

firmed that SST-28 is more abundant in the vitreous fluid than in plasma.

The cause of the decrease in SST-28 within the vitreous fluid of diabetic patients needs to be investigated. Ischemia plays a key role in the pathogenesis of PDR, but it is not a relevant factor in the development of DME. In fact, a significant retinal ischemia was not detected in diabetic patients with DME included in the study. However, intravitreous SST levels detected in DME were similar to those obtained in PDR. In addition, it has been reported that SST is particularly resistant to ischemia (11). Therefore, a significant contribution of ischemia to the deficit of SST production by the retina in diabetic patients seems unlikely. Retinal neurodegeneration is a crucial early event in the pathogenesis of diabetic retinopathy and is involved in the functional deficits in vision that first appear in diabetes even before vascular abnormalities can be appreciated (12). Loss of SST immunoreactivity has been found after degeneration of the retinal ganglion cells (13), and SST levels have been found consistently decreased in the cerebrospinal fluid of patients with various neurodegenerative diseases (14,15). Recently, we have shown that the levels of SST mRNA in the retina were lower in diabetic donors without diabetic retinopathy than in nondiabetic donors, thus suggesting that the reduction of SST mRNA expression in diabetic subjects is an early event in the development of diabetic retinopathy (16). All of these findings suggest that retinal neurodegeneration rather than retinal ischemia is the main reason accounting for the impairment of retinal production of SST by the retina in diabetic patients.

Vascular endothelial growth factor and several proinflammatory cytokines are involved in the pathogenesis of DME (17–20). However, the knowledge of other permeability modulators of the blood-retinal barrier in the setting of DME is still very limited, and there have been no previous studies regarding SST levels in the vitreous of patients with DME. SST exerts inhibitory effects on gastrointestinal secretions and may therefore be beneficial in the treatment of gastrointestinal fistulae (21). In the retina, various ion/water transport systems are located at the apical side of retinal pigment epithelium, adjacent to the subretinal space, and, indeed, a high expression of SST-2 has been shown at this apical membrane of the retinal pigment epithelium (22). Therefore, SST could participate in the balance of fluid and ion transport by these ion transport systems. In addition, SST and SST analogues have anti-inflammatory effects (4–6). Given that SST has anti-edema and anti-inflammatory properties, it could be postulated that its deficit might be involved in the pathogenic events that lead to DME.

Systemic administration of SST analogues has been successfully used as a treatment of DME in isolated cases (23–25), but thus far there are no large series demonstrating its usefulness. The blood-retinal barrier is a limiting factor for systemic administration of drugs that should target the retina. Our findings suggest that intravitreous injection of SST analogues or gene therapy as a replacement treatment would represent a more rational approach to DME treatment.

In conclusion, we have demonstrated that, as occurs in PDR, intravitreal SST-28 levels are significantly decreased in patients with DME. Further studies addressed to clarify the potential contribution of this deficit in the pathogenesis of DME are

needed. Finally, our results support new therapeutic strategies based on intravitreous SST replacement.

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