Fibrinolytic Activity of the Retinae in Streptozotocin-Diabetic Rats

Tatsuro Ishibashi,* Sadami Inoue,† and Kenzo Tanaka†

Fibrinolytic activity of the retinae in control and diabetic rats was assayed quantitatively in twenty male rats made diabetic by giving a single injection of streptozotocin. All these rats were killed at either 3 months or 12 months. Ten saline-injected rats and five rats treated with 3-O-methylglucose and streptozotocin served as controls. As the plasminogen activator activity in diabetic rats maintained for 12 months was significantly lower than that in controls, we postulate that there may be a poor defense mechanism against microthrombus formation in the retinal vasculature of diabetics, which may contribute to the development of diabetic retinopathy. Invest Ophthalmol Vis Sci 26:125-127, 1985

Clinically, the occlusion of retinal vascular beds is an important phenomenon in the development of diabetic retinopathy. We previously suggested that the initial vascular occlusion was due to microthrombus formation. The plasminogen activator is localized mainly in the vascular endothelium and converts plasminogen to plasmin, which in turn acts on fibrin or fibrinogen. In physiologic states, fibrinolysis in vascular beds plays an important role in the resolution of thrombi, and patency of the vasculature is maintained.

We now have studied the fibrinolytic activity of the retinae in control and diabetic rats to determine whether plasminogen activator activity of the retinae would be influenced by diabetes.

Materials and Methods. Twenty male Wistar-King A rats, weighing approximately 200 g, were given streptozotocin intravenously, 65 mg/kg body weight, dissolved in 0.3 ml citrate buffer (pH 4.5), to induce diabetes. Ten control rats of similar body weight were pre-treated with 3-O-methylglucose (3-OMG) before ad- ministration of streptozotocin, thus precluding direct toxicity of streptozotocin; 3-OMG (1.1 mmole/200 g body weight), dissolved in 1 ml 0.9% NaCl was given intravenously over 60 sec, followed by streptozotocin injection.3 The animals were examined every 3 weeks for body weight, blood glucose, and glycosuria. The blood glucose was determined with Dextrostix and glycosuria checked with Tes-Tape. A diagnosis of diabetes was established by persistent hyperglycemia (>300 mg/100 ml), glycosuria, and impaired growth.

All rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (9 mg/100 g body...
weight). One eye of each animal was enucleated immediately, and the anterior segment was removed. After rapid removal of sclera, choroid and retinal pigment epithelium, the remainder of the retina (10–15 mg wet weight) was immersed in 1 ml of ice-cold 2 M NH$_4$SCN (pH 7.4) and stored at -70°C. The retinas were used for the following experiments within 24 hr.

Ten of the twenty diabetic rats were euthanized 3 months after the injection of streptozotocin and the others at 12 months. The ten control rats and five 3-OMG-treated rats were killed 12 months after the injection of the control solution. Since in preliminary experiments (3 control rats) no difference in plasminogen activator levels was observed between the two retinae of each rat, in subsequent experiments, only one retina was used for each quantitative assay for plasminogen activator activity. Extraction of tissue plasminogen activator was carried out with 2 M NH$_4$SCN adjusted to pH 7.4 and stored at -70°C. The retinae were used for the following experiments within 24 hr.

Results. The biochemical data obtained in this experiment are shown in Table 1. The data represent the mean for all the animals at the time of enucleation. Blood glucose in the diabetic groups was significantly higher than that in the control group or that in the 3-OMG-treated group ($P < 0.001$), and blood glucose in the diabetic group maintained for 12 months was lower than that in the diabetic group maintained for 3 months, the decrease being statistically significant ($P < 0.05$). Body weight in the diabetic groups was significantly lower than that in the control group or that in the 3-OMG-treated group ($P < 0.001$). Body weight in the diabetic group maintained for 12 months was higher than that in the diabetic group maintained for 3 months, the increase being statistically significant ($P < 0.01$).

These investigations adhered to the ARVO Resolution on the Use of Animals in Research.

Results. The biochemical data obtained in this experiment are shown in Table 1. The data represent the mean for all the animals at the time of enucleation. Blood glucose in the diabetic groups was significantly higher than that in the control group or that in the 3-OMG-treated group ($P < 0.001$), and blood glucose in the diabetic group maintained for 12 months was lower than that in the diabetic group maintained for 3 months, the decrease being statistically significant ($P < 0.05$). Body weight in the diabetic groups was significantly lower than that in the control group or that in the 3-OMG-treated group ($P < 0.001$). Body weight in the diabetic group maintained for 12 months was higher than that in the diabetic group maintained for 3 months, the increase being statistically significant ($P < 0.01$).

Results of the quantitative assay for plasminogen activator activity of the retinae are summarized in Table 2. Plasminogen activator activity of the retinae was 34.2 ± 3.7 UKmIU/mg protein (mean ± SEM) in the control group, 35.0 ± 2.8 UKmIU/mg protein in the 3-OMG-treated group, 29.8 ± 4.3 UKmIU/mg protein in the diabetic group maintained for 3 months, and 21.9 ± 2.1 UKmIU/mg protein in the diabetic group maintained for 12 months. However, no statistically significant difference was detected between the control group, 3-OMG-treated group or diabetic group maintained for 3 months, nor between diabetic groups maintained for 3 months and for 12 months.

In contrast, in the diabetic group maintained for 12 months, the decrease was statistically significant ($P < 0.05$).

### Table 1. Biochemical data representing the mean for all animals at enucleation time*

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mg/100 ml)</th>
<th>Glycosuria</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (N = 10)</td>
<td>97.0 ± 7.0</td>
<td>(-)</td>
<td>600.8 ± 21.0</td>
</tr>
<tr>
<td>3-OMG-treated group (N = 5)</td>
<td>108.0 ± 11.6</td>
<td>(-)</td>
<td>570.8 ± 23.6</td>
</tr>
<tr>
<td>Diabetic group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months (N = 10)</td>
<td>435.0 ± 14.0±</td>
<td>(++)</td>
<td>235.0 ± 5.7±</td>
</tr>
<tr>
<td>12 months (N = 10)</td>
<td>387.0 ± 10.2±</td>
<td>(+) - (++)</td>
<td>294.9 ± 8.9±</td>
</tr>
</tbody>
</table>

* The values represent mean ± SEM; $N =$ number of rats in each group; $\dagger P < 0.001$ versus control; $\ddagger P < 0.001$ versus 3-OMG-treated; $\dagger\ddagger P < 0.05$ diabetic (3 months) versus diabetic (12 months); and $\ddagger\ddagger P < 0.01$ diabetic (3 months) versus diabetic (12 months).

### Table 2. Results of quantitative assay for plasminogen activator activity of retinae*

<table>
<thead>
<tr>
<th></th>
<th>Plasminogen activator activity (UKmIU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (N = 10)</td>
<td>34.2 ± 3.7</td>
</tr>
<tr>
<td>3-OMG-treated group (N = 5)</td>
<td>35.0 ± 2.8</td>
</tr>
<tr>
<td>Diabetic group</td>
<td></td>
</tr>
<tr>
<td>3 months (N = 10)</td>
<td>29.8 ± 4.3</td>
</tr>
<tr>
<td>12 months (N = 10)</td>
<td>21.9 ± 2.1</td>
</tr>
</tbody>
</table>

* The values represent mean ± SEM; $N =$ number of rats in each group; $\dagger P < 0.05$ control versus diabetic (12 months); and $\ddagger P < 0.05$ 3-OMG-treated versus diabetic (12 months).
Discussion. Increased\(^6\), normal\(^7\), and decreased\(^8\) fibrinolytic activity has been reported in diabetes mellitus. Cash and McGill\(^6\) reported that there was a statistically significant mean shorter euglobulin lysis time in the diabetic group. Tanser\(^7\) found that diabetic patients responded to injected adrenaline with the same increase in blood fibrinolytic activity as nondiabetics. Alfer and Pandolfi\(^8\) reported that the spontaneous fibrinolytic activity of the blood, measured by testing redissolved euglobulin precipitate of citrated plasma on unheated bovine fibrin plates, was more often low in diabetics than in nondiabetic controls, and on stimulation with venous occlusion of the arms for 20 min, the mean fibrinolytic response of the blood was significantly lower in the diabetics. The content of plasminogen activator of the superficial dorsal vein walls, measured according to the histochemical method of Todd as modified by Pandolfi, was more often low in vessels from diabetics.

Thus, there is no agreement about generalized fibrinolytic activity in diabetics. Localized fibrinolytic activity may be more important than generalized fibrinolytic activity with regard to initiation and development of diabetic retinopathy. Localized fibrinolytic activity of the retinae has not been studied previously.

Plasminogen activator activity of the retinae in our diabetic group of rats maintained for 12 months was significantly lower than that in the control group. The retinae of the 3-OMG-treated rats showed no evidence of decreased plasminogen activator activity, thus, direct toxicity of streptozotocin could be ruled out as the cause of the decreased plasminogen activator activity in diabetic rats maintained for 12 months.

The decrease in plasminogen activator that we observed may be the result of low circulating plasminogen activator, the result of localized capillary loss in the retina, the result of decreased plasminogen activator synthesis in the retinal vascular beds, or a combination of these factors. We previously reported that microthrombi, composed mainly of platelets and fibrin strands, could be demonstrated ultrastructurally after 9–12 months.\(^1\) In the vessels in which thrombi were present, there was no definite evidence of loss of endothelial cells or loss of the vascular beds. However, the retinae in the present study could not be examined histologically; in this experiment, we could not determine the cause(s) of the decrease in retinal fibrinolytic activity.

The diabetic group maintained for 3 months showed significantly higher blood glucose and significantly lower body weight than the animals maintained for 12 months and showed only slightly lower plasminogen activator activity than the controls. It appears, therefore, that decreased fibrinolytic activity of the retinae may not be related to the severity of the diabetes, but, rather, to the duration of the diabetes. We are aware that the levels of fibrinolytic activity in the 3-month group cannot be compared with those of the 12-month groups (diabetic or control) because of the possibility that there may be an aging effect. However, even though preliminary, these results point to the duration of diabetes as the major factor in the decrease in plasminogen activator activity.

Recently, several investigators have suggested that thrombus formation is the initial change in retinal vascular occlusion.\(^1\)\(^9\)\(^10\) This decrease in retinal fibrinolytic activity may have a relation to thrombus formation and thus may play a role in the development of diabetic retinopathy.

Key words: fibrinolytic activity, plasminogen activator, retina, streptozotocin-diabetes, rat, radioactivity

Acknowledgments. The authors wish to thank Nino Sor gente, PhD, for his invaluable advice and Ann Dawson for her editorial assistance (Estelle Doheny Eye Foundation).

From the Departments of Ophthalmology* and Pathology,† Faculty of Medicine, Kyushu University, Fukuoka, Japan. Submitted for publication: November 15, 1983. Reprint requests: Tatsuro Ishibashi, MD, Department of Ophthalmology, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan.

References