Renin, Prorenin, and Immunoreactive Renin in Vitreous Fluid From Eyes With and Without Diabetic Retinopathy

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ABSTRACT. Renin, prorenin, and immunoreactive renin were present in vitreous and subretinal fluid of eyes from subjects with and without diabetic retinopathy. Renin substrate, albumin, transferrin, and immunoglobulin G were also found in these ocular fluids. In many samples renin levels were close to the detection limit of the assay. The levels of renin substrate, albumin, transferrin, and immunoglobulin G varied widely among ocular fluid samples, but in each individual sample the levels were, relative to each other, similar to those in plasma. In contrast, the prorenin level in ocular fluid was up to 100 times higher than expected on the basis of the plasma protein content of ocular fluid. Moreover, there was little difference in prorenin

concentrations between samples with low and high plasma protein contents. Prorenin, relative to albumin and other plasma proteins, was higher in vitreous fluid from eyes with proliferative diabetic retinopathy complicated by traction retinal detachment than in eyes of nondiabetic subjects with spontaneous retinal detachment. It appears that prorenin (and possibly renin) in ocular fluid is controlled by an active and specific process, possibly local synthesis within the eye. In view of the vascular actions of angiotensin II, an intraocular renin-angiotensin system may play a role in diabetic retinopathy. (*J Clin Endocrinol Metab* 68: 160, 1989)

THE KIDNEY secretes both renin and prorenin, an inactive precursor of renin, into the circulation. Plasma of nephrectomized patients contains little or no renin, but it does contain prorenin (1, 2), sometimes in concentrations as high as those in normal individuals. It thus appears that extrarenal production can make a major contribution to the level of prorenin in plasma, whereas most, if not all, renin in plasma is secreted by the kidneys. Synthesis of renin or prorenin and other components of the renin-angiotensin system is known to occur at various extrarenal sites, for instance adrenal (3, 4), pituitary (3, 4), testis (3, 4), brain (5), and ovary (6-8). Cultured human chorionic cells (9) and ovarian thecal cells (8) release prorenin into the medium, and there is good evidence that in women with hyperstimulated cycles and during pregnancy, the ovary, probably the corpus luteum, releases prorenin into plasma (7, 10).

A common feature of the organs in which synthesis of renin or prorenin occurs is their extensive vascularization (11). The eye, particularly the retina and uveal tract, is a highly vascularized organ. Angiotensin II-binding sites have been found in retinal blood vessels (12), and

transvitreal infusion of angiotensin I and II produces constriction of the retinal arteries (13). The retina contains angiotensin-converting enzyme activity (14), and this enzyme is also found in aqueous fluid (15). Here we report measurements of renin, prorenin, immunoreactive renin, renin substrate, and various plasma proteins in aqueous, vitreous, and subretinal fluid. The ocular fluid samples were obtained at the time of cataract extraction or vitrectomy, and the protein concentrations in these samples were compared with those in simultaneously obtained plasma. Our study included eyes affected by proliferative diabetic retinopathy, because the renin-angiotensin system has been implicated in neovascularization (16).

Subjects and Methods

Nondiabetic subjects

Aqueous fluid was collected at the time of cataract extraction from 21 subjects (15 women and 6 men; mean age, 68 yr; range, 26-86 yr). Four subjects were receiving a diuretic and 6 a β -adrenergic antagonist.

Vitreous fluid aspirates were obtained from 16 subjects (8 women and 8 men; mean age, 52 yr; range, 20-82 yr). The samples were collected at the time of pars plana vitrectomy, which was performed because of recurrent retinal detachment due to proliferative vitreoretinopathy. Four subjects were receiving a diuretic, in 3 of them combined with a β -adrenergic

Received May 18, 1988.

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antagonist.

Subretinal fluid was obtained from 18 subjects (8 women and 10 men; mean age, 59 yr; range, 8–76 yr) with rhegmatogenous retinal detachment, which is a type of retinal separation precipitated by a hole or tear in the retina. In this type of detachment fluid accumulates between the retinal pigment epithelial layer and the neural retina. The retinal detachments had occurred between 1 day and 3 months (median, 1 week) before subretinal fluid collection. Three subjects were receiving a diuretic, in 2 of them combined with a β -adrenergic antagonist.

Diabetic subjects

Vitreous fluid was obtained from 15 diabetic subjects with proliferative diabetic retinopathy (8 women and 7 men; mean age, 51 yr; range, 28–71 yr). Vitrectomy was performed because of traction retinal detachment. The duration of diabetes ranged from 6–32 yr. Twelve subjects were receiving insulin, 5 were receiving a diuretic, and 1 was receiving a β -adrenergic antagonist.

Aqueous fluid can only be collected at the time of cataract extraction. In diabetic subjects, however, this procedure may stimulate proliferative retinopathy. Cataract extraction is, therefore, not performed in eyes affected by proliferative diabetic retinopathy. Consequently, aqueous fluid could not be collected from such eyes. We also were unable to collect subretinal fluid from diabetic subjects with traction retinal detachment, because drainage of subretinal fluid is rarely performed in these subjects, and if it is performed, the approach is via the transvitreal route, so that the sample is heavily contaminated with material from the vitreous. In subjects with a rhegmatogenous retinal detachment, subretinal fluid is removed via the transscleral route, where no such contamination occurs.

Collection of ocular fluid samples

Approximately 0.1 mL aqueous fluid was collected with a tuberculin syringe and a 25-gauge needle. The needle was introduced at the limbus of the cornea through the groove of the cataract incision. A 0.3- to 1.0-mL sample of vitreous fluid was aspirated before substitution fluid was infused into the vitreous. Subretinal fluid was aspirated transsclerally, after local diathermic coagulation of the choroid.

The ocular fluid samples were free of macroscopically visible blood and were frozen at -70°C immediately after collection. A peripheral venous blood sample was drawn simultaneously with the collection of ocular fluid. Blood for determination of renin, prorenin, immunoreactive renin, renin substrate, albumin, transferrin, and immunoglobulin G (IgG) was collected in tubes containing 0.1 vol 0.13 mol/L trisodium citrate. The blood was immediately centrifuged at $3000 \times g$ for 10 min at room temperature, and 1-mL aliquots of plasma were stored at -70°C . Blood for determination of angiotensin II was collected in prechilled tubes containing 0.1 vol 0.06 mmol/L pepstatin-A, 0.125 mol/L disodium EDTA, and 0.025 mol/L phenantroline in order to block renin, angiotensin-converting enzyme, and angiotensinases, respectively. The blood samples were immediately centrifuged at $3000 \times g$ for 10 min at 4°C , and 2-mL aliquots of plasma were stored at -70°C .

Analytical methods

Renin was measured in duplicate by enzyme kinetic assay, in which the samples were incubated at 37°C and pH 7.5 with saturating amounts of sheep renin substrate in the presence of inhibitors of angiotensinases and angiotensin-converting enzyme. The generated angiotensin I was quantitated by RIA (17). For measuring prorenin in plasma, prorenin was converted into renin by incubation with Sepharose-bound trypsin (0.25 mg/mL) for 48 h at 4°C .

Previous studies, including measurements of total immunoreactive renin (renin plus prorenin), indicated that the prorenin to renin conversion in plasma is complete after incubation with the immobilized trypsin under these circumstances and that destruction of renin or prorenin does not occur (18). Experiments in which known quantities of purified human kidney renin were added to ocular fluid demonstrated that in some samples destruction of renin did occur with this method. This destruction might be due to the low content of serine protease inhibitors in ocular fluids compared to that in plasma. Therefore, in ocular fluid we chose to use plasmin to convert prorenin into renin (17, 19). For this purpose the sample was incubated with plasmin at a final concentration of $0.5 \mu\text{mol/L}$ for 48 h at 4°C before the assay.

Comparison of the results of the enzyme kinetic assay in plasmin-activated ocular fluid samples with the results of the assay of total immunoreactive renin in nonactivated samples demonstrated that the conversion of plasmin was complete without any loss of prorenin or renin; the specific enzymatic activity of plasmin-activated prorenin in ocular fluid samples was not different from the specific activity of purified kidney renin and plasma renin (see *Results*). Plasmin at the concentration mentioned above cannot be used to activate prorenin in native whole plasma because of its high content of plasmin inhibitors.

The concentrations of renin and prorenin measured by the enzyme kinetic assay were expressed as milliunits per L using the WHO human kidney renin standard 68/356 (WHO International Laboratory for Biological Standards, London, United Kingdom) as reference standard. The lower limit of detection was 0.5 mU/L , and the interassay variability at low concentrations of renin or prorenin ($2\text{--}5 \text{ mU/L}$) was 11% for both renin and prorenin.

Immunoreactive renin was measured in duplicate with a sandwich assay (18, 20) using the monoclonal antibodies R 3-27-6 and R 3-36-16 (Ciba-Geigy, Basel, Switzerland). The two monoclonal antibodies recognize different epitopes of the renin molecule and react equally well with human kidney renin and chorionic cell culture prorenin. The assay was carried out in polystyrene tubes (Star Tubes, code 4-70319, Nunc, Roskilde, Denmark). The inner surface of these tubes was coated with antibody R 3-27-6 (21). Immunoreactive renin in the assay sample is quantitatively bound to this antibody. The amount of solid phase bound immunoreactive renin was measured with antibody R 3-36-16, which had been radiolabeled with ^{125}I . The results of this assay were expressed as nanograms per L using highly purified human kidney renin (Ciba-Geigy) as a standard. One milliunit of the WHO human kidney renin standard corresponded to 1.41 ng Ciba-Geigy standard. The lower limit of

detection was 5 ng/L, and the interassay variability was 8%.

The concentration of renin substrate was determined as the maximum quantity of angiotensin I that was generated during incubation at 37°C and pH 7.5 with an excess of purified active human kidney renin in the presence of inhibitors of angiotensinases and angiotensin-converting enzyme (18). The lower limit of detection was 1 nmol/L, and the interassay variability was 10%.

Immunoreactive angiotensin II was measured by RIA after Sep-Pak (Waters, Milford, MA) extraction of the sample (22). The lower limit of detection was 2 pmol/L, and the interassay variability was 15%.

Albumin, transferrin, and IgG were measured by single radial immunodiffusion (LC and NOR-Partigen plates, Behringwerke, Marburg, West Germany) according to the method of Mancini *et al.* (23).

Data analysis

Plasma proteins enter the vitreous mainly by diffusion. One of the reasons why the concentrations of these proteins are low in vitreous fluid is that they have to cross a relatively impermeable barrier. Breakdown of this so-called blood-retinal barrier leads to increased diffusion of plasma proteins into the eye. The rate of diffusion of a given protein is related to its molecular size and plasma concentration. In accordance with this is the fact that the concentrations of the different proteins relative to each other are similar in plasma and vitreous fluid (24, 25).

Thus, unless certain specific uptake processes exist, for which in the eye no evidence is available with regard to any of the proteins mentioned in this paper, one would expect a relatively high intraocular albumin concentration (due to partial breakdown of the blood-retinal barrier) to be accompanied by a proportionally high concentration of plasma proteins of comparable size. Therefore, we chose to take the vitreous fluid/plasma concentration ratio of albumin as an index of the integrity of the blood-retinal barrier, an abnormally high ratio being an indication of breakdown of this barrier. By multiplying this ratio with the level of a given protein in plasma, the level of this protein in ocular fluid can be estimated, assuming that, as mentioned above, this protein is transferred from the blood into the vitreous and *vice versa* by mechanisms that are qualitatively and quantitatively the same as those for the transfer of albumin. For example, for renin substrate the calculation would be as follows:

$$\left[\text{RS}_{\text{oc}} \right] = \left[\frac{\text{ALB}_{\text{oc}}}{\text{ALB}_{\text{pl}}} \right] \cdot \left[\text{RS}_{\text{pl}} \right]$$

in which RS is renin substrate, ALB is albumin, oc is ocular fluid, pl is plasma, and brackets denote the concentration.

If our assumptions are correct, the calculated concentrations should be equal or at least closely correlated to the actually measured concentrations. Therefore, the two sets of data were analyzed by linear regression.

For analyzing differences between diabetic and nondiabetic subjects unpaired *t* tests were performed after logarithmic

transformation of the data. Values were considered significant if $P < 0.05$.

Results

Nondiabetic subjects

The levels of renin in many vitreous and aqueous fluid samples were at or below the detection limit of the assay (0.5 mU/L), which is less than 5% of the level in plasma. In subretinal fluid the renin level was about 20% of that in plasma (Table 1). Prorenin was detectable in all samples of vitreous and aqueous fluid; its level in vitreous fluid was about 20% and in aqueous fluid about 5% of that in plasma. In subretinal fluid the prorenin level was as high as that in plasma. Renin and prorenin concentrations in the fluid compartments of the eye were in the order: subretinal fluid > vitreous fluid > aqueous fluid. The levels of renin substrate in subretinal, vitreous, and aqueous fluid were 10%, 5%, and 0.5% of those in plasma, respectively. Thus, they too were in the order: subretinal fluid > vitreous fluid > aqueous fluid. This was also true for the levels of albumin, transferrin, and IgG (Table 2). There was no correlation between the levels in ocular fluid and those in plasma for any of the proteins.

As described under *Data analysis* above, a theoretical concentration in ocular fluid for each protein was predicted based on the albumin content of the sample. For renin substrate, transferrin, and IgG the calculated and measured values were linearly correlated in both vitreous and subretinal fluid, and the slopes of these correlation lines were not significantly different from 1.0 (Tables 3 and 4). Renin substrate and transferrin concentrations in ocular fluid could, in fact, be accurately predicted by these calculations. The IgG level measured was systematically about 2 times lower than that calculated, which may be due, at least in part, to its larger molecular size compared to those of albumin and the other proteins.

For prorenin the findings were different. The prorenin levels in both vitreous and subretinal fluid varied much less than the levels of the other proteins (Tables 1 and 2). Furthermore, calculating prorenin concentrations on the basis of the albumin content of the sample yielded much lower (down to 1/100th) values than those actually measured, particularly in samples with a low plasma protein content (intact blood-retinal barrier). The prorenin level in subretinal fluid was higher than that in vitreous fluid, even when corrections were made for the higher plasma protein content in subretinal fluid samples (Fig. 1). In both vitreous and subretinal fluid the slopes of the regression lines describing the correlation between the measured and calculated prorenin concentrations were significantly different from 1.0, thereby indicating the different behavior of prorenin compared to albumin and other plasma proteins.

TABLE 1. Levels of prorenin, renin, and renin substrate in ocular fluids

	n	Prorenin (mU/L)		Renin (mU/L)		Renin substrate (nmol/L)	
		Eye	Plasma	Eye	Plasma	Eye	Plasma
Non-diabetic subjects							
Aqueous vs. plasma	21	4.4 2.0-8.7	163 36.7-453	<0.5 ND-0.5	9.8 1.5-62.9	5.1 1.6-15.4	1080 898-1430
Vitreous vs. plasma	16	34.5 17.4-61.9	174 67.0-396	<1.0 ND-2.8	17.3 3.7-51.0	54.5 3.0-630	1120 791-3030
Subretinal vs. plasma	18	132 36.8-305	128 65.1-251	2.4 0.9-6.4	14.3 4.3-105	107 10-1430	1230 841-2250
Diabetic subjects							
Vitreous vs. plasma	15	61.0 19.0-172	357 121-679	<2.0 ND-3.5	17.7 4.4-127	61.0 7.0-1000	1030 628-2040

Shown are the geometric mean and range. ND, Not detectable. In vitreous fluid and plasma the levels of prorenin, but not those of renin substrate, were higher in diabetic than in nondiabetic subjects ($P < 0.01$).

TABLE 2. Levels of albumin, IgG, and transferrin in ocular fluids

	n	Albumin (g/L)		IgG (g/L)		Transferrin (g/L)	
		Eye	Plasma	Eye	Plasma	Eye	Plasma
Nondiabetic subjects							
Aqueous vs. plasma	21	0.19 0.06-0.45	33.1 28.3-39.4	ND	ND	ND	ND
Vitreous vs. plasma	16	1.55 0.11-20.2	33.0 22.9-42.5	0.19 0.03-0.70	11.0 6.61-14.4	0.18 0.02-1.74	2.44 1.60-2.99
Subretinal vs. plasma	18	3.03 0.39-28.5	35.4 29.2-41.9	0.48 0.07-5.10	11.2 7.0-18.2	0.45 0.06-3.97	2.57 2.08-3.44
Diabetic subjects							
Vitreous vs. plasma	15	1.51 0.32-17.1	29.2 21.7-36.8	0.19 0.04-0.96	9.2 3.88-16.7	0.14 0.06-0.37	2.06 1.50-2.70

Shown are the geometric mean and range. The levels of albumin, IgG, and transferrin in both vitreous and plasma did not differ between diabetic and nondiabetic subjects. ND, Not done.

TABLE 3. Correlations between the measured and calculated concentrations of proteins in vitreous fluid

Protein	Regression line	r	P
Prorenin			
Nondiabetic subjects	$y = 0.015^a x + 1.523$	0.05	NS
Diabetic subjects	$y = 0.328^a x + 1.370$	0.64	<0.05
Renin substrate	$y = 1.024x - 0.028$	0.97	<0.0001
IgG	$y = 1.063x - 0.344$	0.91	<0.0001
Transferrin	$y = 0.899x + 0.066$	0.89	<0.0001

y is the log (measured concentration); x is the log (calculated concentration). For renin substrate, IgG, and transferrin, data from diabetic and nondiabetic subjects were combined because no differences were found for these proteins between the two groups (Tables 1 and 2).

^a Slope different from 1.0, $P < 0.0001$.

The data on prorenin shown in Tables 1, 3, and 4 and Figs. 1-3 were obtained by the enzyme kinetic assay. That prorenin measured by this assay is, in fact, prorenin is supported by the excellent agreement with the measurements of immunoreactive renin (Fig. 4). The mean specific enzymatic activity of *in vitro* activated prorenin was 0.7 ± 0.2 ($\pm$ SD) mU/ng ($n = 9$) in vitreous fluid and 0.6 ± 0.2 mU/ng ($n = 10$) in subretinal fluid. These values are not different from the specific activity of renin

TABLE 4. Correlations between the measured and calculated concentrations of proteins in subretinal fluid

Protein	Regression line	r	P
Prorenin	$y = 0.271^a x + 1.839$	0.61	<0.01
Renin substrate	$y = 0.975x + 0.064$	0.98	<0.0001
IgG	$y = 0.941x - 0.302$	0.99	<0.0001
Transferrin	$y = 0.934x + 0.262$	0.94	<0.0001

y is the log (measured concentration); x is the log (calculated concentration).

^a Slope different from 1.0, $P < 0.0001$.

from plasma and kidney (18).

The immunoreactive angiotensin II level was 11.1 ± 1.8 pmol/L in vitreous fluid ($n = 12$) compared to 17.5 ± 1.3 pmol/L in plasma. In subretinal fluid ($n = 15$) it was 14.8 ± 1.6 pmol/L compared to 23.9 ± 2.0 pmol/L in plasma.

Diabetic subjects

The results of the renin substrate, albumin, transferrin, and IgG measurements in vitreous fluid and plasma of the diabetic subjects were similar to those in the nondiabetic subjects (Tables 1 and 2). The levels of renin

and prorenin in vitreous fluid were higher in the diabetic than in the nondiabetic subjects. Prorenin was also higher when allowance was made for differences in the

FIG. 1. Mean ($\pm$ SE) prorenin/albumin concentration ratio in ocular fluids and plasma of nondiabetic subjects.

plasma protein content of the samples. In the diabetic subjects the prorenin concentration of vitreous fluid correlated with the plasma prorenin concentration ($r = 0.78$; $n = 15$; $P < 0.001$). In the nondiabetic subjects there was no significant correlation between the levels of prorenin in vitreous fluid and plasma.

As in the nondiabetic subjects, prorenin in vitreous samples with low plasma protein content was much higher (up to 25 times) than expected on the basis of the albumin content of the samples.

Immunoreactive angiotensin II was 9.0 ± 2.5 pmol/L in vitreous fluid ($n = 15$) compared to 9.9 ± 2.9 pmol/L in plasma.

Discussion

The levels of albumin (mol wt, 69K), transferrin (mol wt, 90K), IgG (mol wt, 150K), and renin substrate (mol wt, 65K) in vitreous fluid differed widely from sample to sample, but in each individual sample the levels were, relative to each other, comparable to those in plasma. The IgG level in vitreous fluid, relative to that of albumin, was systematically somewhat lower than that in plasma, probably due to its larger molecular size (25). These results are in agreement with earlier findings that most soluble protein in the vitreous is derived from plasma (24–26).

The plasma protein content of normal vitreous fluid has been estimated to be 0.5–2% of that in plasma (25, 26). In our study vitreous fluid from eyes with recurrent retinal detachment due to proliferative vitreoretinopathy contained higher levels of plasma proteins. These higher values probably reflect partial breakdown of the blood-

FIG. 2. Measured vs. calculated concentrations of prorenin (left) and renin substrate (right) in vitreous fluid. For an explanation of the calculation see text (Data analysis). The slopes and significance levels of the correlations are given in Table 3. O, Diabetic; ●, nondiabetic.

FIG. 3. Measured vs. calculated concentrations of prorenin (left) and renin substrate (right) in subretinal fluid. For an explanation of the calculation see text (*Data analysis*). The slopes and significance levels of the correlations are given in Table 4.

FIG. 4. Total renin (prorenin plus renin) measured by enzyme kinetic assay vs. immunoreactive renin ($r = 0.97$; $P < 0.0001$). O, Plasma; ■, subretinal fluid; ●, vitreous fluid; ▲, aqueous fluid; +, WHO human kidney renin standard.

retinal barrier in such eyes (27).

The blood-retinal barrier is formed by the tight junctions between the endothelial cells of the retinal capillaries and the tight junctions between the retinal pigment epithelial cells, the latter restricting the transfer of plasma proteins escaping from the capillaries of the choroid (28). Proteins from plasma may enter the vitreous as a result of focal cellular necrosis, opening of the intercellular junctions, or vesicular transport and formation of transcellular channels. The rate of diffusion

of proteins through such discontinuities in the blood-retinal barrier depends upon the concentration gradient across this barrier, the molecular size of the proteins, and the number and area of discontinuities. Our results are in accordance with the contention that diffusion through these pores is the main mechanism of transfer of plasma proteins to the vitreous.

This process, however, does not appear to hold true for prorenin (mol wt, 54K). The concentration of prorenin, relative to that of albumin, was much higher in vitreous fluid than in plasma, and the prorenin level in vitreous fluid also was little influenced by its plasma protein content. Thus, prorenin may enter the vitreous by a mechanism different from that of albumin and other plasma proteins. This mechanism is selective for prorenin and may involve an active process. Receptor-mediated transcellular transport is such a selective mechanism, but as yet there is no evidence for the existence of cell membrane receptors for prorenin. Our findings raise the possibility that not all prorenin in the vitreous is derived from plasma; some of it may be produced in the eye.

As described under *Data analysis* above, the vitreous level of prorenin that has crossed the blood-retinal barrier by passive diffusion in the same way as albumin can be estimated by multiplying the vitreous/plasma concentration ratio of albumin by the plasma prorenin level. By subtracting this calculated level of plasma-derived prorenin from the level actually measured, we estimated the level of prorenin that entered the vitreous by some process different from diffusion out of the circulation. In most samples of vitreous fluid the estimated level of prorenin that had entered the vitreous by such a diffu-

sion-independent process was more than 4 times higher than the estimated level of prorenin that had entered the vitreous by passive diffusion from blood. Thus, relative to the total amount of prorenin in vitreous fluid, the contribution of plasma-derived prorenin crossing the blood-retinal barrier merely by diffusion appears to be small. The implicit assumption underlying these calculations is that albumin and prorenin leave the vitreous in the same way, that is by free diffusion into the aqueous fluid (29), where the concentrations of these proteins were much lower than those in the vitreous.

The concentrations of albumin, transferrin, IgG, and renin substrate were 2–3 times higher in subretinal fluid than in vitreous fluid. This was to be expected, since subretinal fluid from eyes with retinal detachment is more or less a concentrate of vitreous fluid (27). Vitreous fluid enters the subretinal space through the hole(s) of the retina, and water is actively absorbed from the subretinal space by the retinal pigment epithelium. Again, the findings for prorenin were different. The concentration of prorenin, relative to that of albumin, was much higher in subretinal fluid than in plasma, particularly in subretinal samples with low plasma protein concentrations. Moreover, relative to levels of albumin and other plasma proteins, the prorenin concentration was 2 times higher in subretinal than in vitreous fluid. If it is assumed, on the basis of the evidence discussed above, that most of the prorenin in the vitreous is not derived from plasma but is produced in the eye, the difference in prorenin content between subretinal and vitreous fluid may suggest that the subretinal compartment is closer to the site of prorenin production.

Not only were the prorenin concentrations of vitreous and subretinal fluid higher than expected, so too were the renin (mol wt, 48K) concentrations. The data on renin, however, are more difficult to interpret than those on prorenin because in many samples renin was at or below the detection limit of the assay, and some prorenin to renin conversion may have occurred during storage and handling of the samples. Even as little as 1% conversion will result in a large percent increase in renin in these samples.

Immunoreactive angiotensin II also was found in samples of vitreous fluid, in concentrations comparable to those in plasma. Further work is needed to determine its origin.

That the levels of albumin and IgG in vitreous fluid from eyes affected by proliferative diabetic retinopathy were higher than the levels in normal eyes can be explained by the increased permeability of the blood-retinal barrier in this condition (30). The higher vitreous level of prorenin, relative to those of albumin and other plasma proteins, in diabetic subjects compared to nondiabetic subjects is more difficult to explain. The same arguments

in favor of the hypothesis that, generally, diffusion from the blood contributes little to the total amount of prorenin in the vitreous apply to both diabetic and nondiabetic subjects. Therefore, it seems unlikely that the higher level of prorenin in vitreous fluid of the diabetic subjects (2 times that in nondiabetic subjects) was caused by the higher level in plasma (also 2 times that in nondiabetic subjects). It might be the other way around; increased release or leakage of prorenin from the eye affected by proliferative diabetic retinopathy may contribute to the increased prorenin level in plasma.

This possibility is further supported by the finding that, in contrast with other proteins, the plasma concentration of prorenin in diabetic subjects correlated significantly with the concomitant vitreous prorenin concentration. Considering the fact that in some diabetic subjects the blood-retinal barrier for plasma proteins was still relatively intact (low vitreous/plasma albumin concentration ratio), whereas in others it was extremely leaky, no such correlation was to be expected if diffusion from plasma into the vitreous was the main mechanism of transfer of prorenin.

An elevated plasma prorenin level in diabetic subjects has been found to be associated with microvascular complications, including retinopathy (31). Evidence is accumulating that neovascularization is initiated by diffusible chemical factors arising from ischemic areas of the retina (32). Renin and angiotensin have been found in cultured neuronal and glial cells from rat brain (33, 34); both cell types are abundantly present in the retina. Angiotensin II acts on vascular tone and has mitogenic and trophic actions on vascular smooth muscle and other cells (35). In fact, it has been reported to promote neovascularization (16). An intraocular renin-angiotensin system may, therefore, play a role in proliferative diabetic retinopathy.

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