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Metabolism of Glomerular Basement Membrane in Normal, Hypophysectomized, and Growth-Hormone-Treated Diabetic Rats

ALLURU S. REDDI

Department of Medicine, Division of Nephrology, UMDNJ-New Jersey Medical School, 100 Bergen Street, Newark, New Jersey 07103

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The *in vivo* synthesis of the renal glomerular basement membrane (GBM) collagen was studied in normal, hypophysectomized (hypox), diabetic, and growth-hormone (GH)-treated diabetic rats, using tritiated proline (L[2,3,^{3}H]proline) as a radioisotopic precursor.

After the injection of tritiated proline, all groups of rats were sacrificed at various time intervals and the specific activities of proline and hydroxyproline of GBM were determined, the latter being used as a measure of GBM collagen synthesis. A significant decrease in both proline and hydroxyproline specific activities were observed in GBM of hypox rats at all periods of study. Administration of GH to hypox rats returned the GBM collagen synthesis to normal. Diabetic GBM had higher proline and hydroxyproline specific activities when compared to normal rats. Treatment of diabetic rats with GH for 10 days further increased both proline and hydroxyproline specific activities when compared either to diabetic or normal rats treated with GH. The activity of glucosyltransferase, an enzyme involved in the biosynthesis of the disaccharide unit of GBM collagen was found to be decreased in glomeruli of hypox rats. In contrast, the activity of *N*-acetyl-β-glucosaminidase, a glycoprotein-degrading enzyme, was found to be significantly increased in hypox rats. GH treatment restored both enzyme activities to normal. The results of the present study show that GBM collagen synthesis is decreased in hypox rats and increased in diabetic rats. GH treatment not only normalized GBM collagen synthesis in hypox rats but also caused significant increase in diabetic rats. This suggests that the renal GBM metabolism is influenced by GH, and this may be of particular significance in view of GH involvement in diabetic microvascular complications.

INTRODUCTION

In recent years the nature of the renal glomerular basement membrane (GBM) has been the subject of numerous investigations both in health and disease (Kefalides, 1978; Lubec, 1980). In diabetes mellitus, the renal glomerulus is characterized not only by thickening of the GBM, but also changes in its composition and metabolism (Reddi, 1978; Brownlee and Cerami, 1981). Although several reports and reviews have been published, the pathogenesis of these changes is not clearly understood (Spiro, 1976; Camerini-Davalos *et al.*, 1977; Tchbroutsky, 1979). Lundbaek and associates (Lundbaek *et al.*, 1970, 1980) proposed that elevated growth hormone (GH) levels in juvenile diabetes (Type I) might be involved in the pathogenesis of vascular complications. This hypothesis was based on observations that GH levels are elevated in Type I diabetic patients (Lundbaek *et al.*, 1970, 1980; Hansen, 1972) and that hypophysectomy could inhibit the development of diabetic retinopathy (Luft and Guillemin, 1974). Additional evidence in support of GH hypothesis came from studies that GH-deficient dwarfs with glucose intolerance do not develop diabetic vascular complications (Merimee *et al.*, 1973). Osterby *et al.* (1978) provided further evidence that administration of GH to a small number of streptozotocin diabetic rats produced a 2½-fold

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increase in GBM thickness when compared to saline-treated diabetic rats. However, further extension of these studies failed to confirm this report (Seyer-Hansen *et al.*, 1981).

Although GH has been implicated in the pathogenesis of diabetic microvascular complications, its role on GBM metabolism has not been studied either in normal or diabetic animals. The present paper deals with the synthesis of GBM collagen in normal, hypophysectomized, diabetic, and GH-treated diabetic rats. The results indicate that the synthesis of GBM collagen is decreased in hypophysectomized rats and increased in diabetic rats. Administration of GH to hypophysectomized rats restored GBM collagen to normal. In diabetic rats, GH treatment resulted in further enhancement of GBM collagen synthesis.

MATERIALS AND METHODS

Animals. Normal and hypophysectomized (hypox) male albino rats weighing between 60 and 75 g were purchased from Charles River Breeding Laboratories. Some normal rats were fasted overnight and made diabetic by intraperitoneal injection of streptozotocin in the amount of 70 mg/kg in citrate buffer, pH 4.5. These rats were divided into two groups. One group was treated intraperitoneally with rat GH (100 μg/100 g) daily for 10 days, 3 weeks after induction of diabetes. The other group of diabetic rats was treated with 0.9% saline. Another group of age-matched normal rats was treated intraperitoneally with rat GH (100 μg/100 g) for 10 days.

All groups of rats (4–5 weeks following hypophysectomy or induction of diabetes) were injected with a single intraperitoneal dose of [^{3}H]proline (L[2,3,^{3}H]proline; sp act 29.8 Ci/mmole) in approximately 0.5 ml of 0.9% saline. Each rat received 140 μCi/100 g body wt at morning hours. The normal, hypox, and GH-treated hypox rats were killed at 2, 4, 24, 48, 120, and 188 hr, whereas the diabetic, GH-treated diabetic, and GH-treated normal rats were sacrificed at 24 and 48 hr. These two time intervals for the latter groups of rats were selected because the protein synthesis in diabetic rats was maximum at 24 hr and reached a plateau at 48 hr (Reddi, submitted for publication).

At the time of sacrifice, each rat was anesthetized with Nembutal (5 mg/100 g), bled from the abdominal aorta, and the kidneys were rapidly removed, decapsulated, cortices separated, and pooled from a group of two to five rats.

Preparation of glomeruli. The procedure for the isolation of glomeruli is that of Krakower and Greenspon (1951). The cortices were minced with a razor blade and scissors to a paste-like consistency and the material gently pushed through a 170-mesh (90 μm) stainless-steel sieve while washing repeatedly with cold 0.15 *M* NaCl.The bottom of the sieve was scraped with a spatula for maximum recovery of the tissue material. The suspension was allowed to sediment under gravity in plastic centrifuge tubes for 1 hr. The supernatant was removed; 30 ml of cold 0.15 *M* saline was added to resuspend the pellet, and the glomeruli were allowed to settle down. In this way, the glomerular pellet was washed four times. Examination of the pellet under the light microscope consistently revealed a glomerular preparation of 95–97% purity in both normal and hypox rats. The purity of glomeruli obtained was comparable with procedures using sequential sieving through stainless steel meshes of pore size 90 and 63 μm or glomeruli obtained by Ficoll gradient (Grant, Harwood and Williams, 1975).

Preparation and hydrolysis of basement membranes. The glomeruli from each

preparation were suspended in 10 ml of cold 0.15 *M* NaCl and subjected to 10 bursts, each of 1 min duration with the use of an Artek/Fisher sonic dismembrator, at a relative output of 50%, allowing 30-sec intervals for cooling. The basement membrane was then settled by centrifugation at 1000*g* for 15 min at 4°C. The supernatant was discarded. To the basement membrane fraction was added 25 μm of unlabeled proline to dilute any possible radioactivity present as free labeled proline. Six milliliters of 1 *M* NaCl were added, resuspended, and centrifuged as described above. The washings of the basement membrane fractions with 1 *M* NaCl were repeated three times followed by five times with distilled water. Basement membrane samples were initially dissolved in 1.5 ml of hot HCl, transferred quantitatively into 5-ml ampules, sealed, under nitrogen and hydrolyzed at 105°C for 24 hr. The hydrolysates were then evaporated to dryness and the final residues dissolved in 2 ml of distilled water for proline and hydroxyproline determination and radioactivity.

Assay of proline and hydroxyproline. Both specific activity and quantitative measurement of proline and hydroxyproline were determined according to the procedure of Rojkind and Gonzalez (1974). This method is based on the oxidation of proline and hydroxyproline and their conversion, respectively, to pyrroline and pyrrole, followed by separate extractions with toluene.

Separate aliquots of toluene extractions were used for specific activity and quantitative measurements. Standards of proline and hydroxyproline were run simultaneously with the samples. The recovery of [^{3}H]proline after oxidation and extraction three times with toluene was 90% in our hands. Less than 1% of the labeled hydroxyproline was recovered in the proline extraction (toluene) layer.

Radioactivity of proline and hydroxyproline samples was determined in a liquid scintillation counter (Nuclear Chicago, series 720), using 3× scintillation fluid (0.3 g of 1,4-bis-2-(5-phenyloxazolyl) benzene, 12 g of 2,5-diphenyloxazole dissolved in 1 liter of toluene). All counts were converted to disintegrations per minute (dpm) with the appropriate efficiency corrections. The values are expressed as dpm per minute per micromole of either proline or hydroxyproline.

Determination of tissue proline specific activity. Rats were injected with [^{3}H]proline (140 μCi/100 g) intraperitoneally and sacrificed at various time intervals up to 188 hr. Kidneys were isolated, cortices separated, and cortices from two rats combined. They were homogenized in cold 0.15 *M* sodium chloride (20% homogenate), and the homogenates were deproteinized with 3 vol of 10% TCA. The sediment was extracted with 1 vol of TCA and supernatants combined. The TCA was removed by ether extraction three times and the aqueous phase was passed through Dowex 50 column (H^+). The column was washed with 100 ml of distilled water and eluted two times with 5-ml portions of 1.5 *M* NH_4OH. The eluate was evaporated overnight at 60–70°C and the residue was dissolved in 0.5 ml of NH_4OH and 0.5 ml of distilled water. The pH was adjusted between 6 and 7 and the sample was made up to 2 ml with distilled water. Proline was determined according to the procedure of Rojkind and Gonzalez (1974).

Expression of results. Since the specific activity of proline in tissue was found to be substantially lower in normal than in hypox rats, appropriate correction factor was obtained from the geometrical integration of the specific activity of the tissue-free proline. This is derived from the area under the tissue-free proline specific activity curves and the ratio obtained by dividing the hypox into normal. This correction factor was used to multiply the proline and hydroxyproline spe-

cific activity of GBM of normal rats. A similar correction factor was obtained from the area under the tissue-free proline specific activity curve between normal and GH-treated hypox or diabetic rats.

Determination of glucosyltransferase activity. The activity of this enzyme was determined using galactosylhydroxylysine acceptor obtained from commercially available marine sponge after alkaline hydrolysis and purification by ion-exchange chromatography and gel filtration (Smith, 1978). Glomeruli from individual rats were homogenized in 0.25 ml of 0.15 *M* Tris-acetate buffer, pH 6.8, containing 0.002 *M* 2-mercaptoethanol and centrifuged at 10,000*g* for 10 min. The supernatant was separated and used for the enzyme assay. The reaction mixture contained in a total volume of 0.120 ml: 50 μl of buffer, 50 μl of enzyme preparation, 10 μl of 6 m*M* acceptor (galactosylhydroxylysine), 10 μl of 0.25 *M* manganese, and 10 μl containing 0.04 μmole of [UDP-^{14}C]glucose (sp act 4 μCi/μmole). The UDP-glucose was evaporated to dryness under nitrogen prior to the addition of enzyme, substrate, and manganese. The amount of glomerular protein ranged from 40 to 120 μg. After incubation for 2 hr at 37°C, the reaction was stopped by the addition of 0.6 ml of cold 1% phosphotungstic acid in 0.5 *M* HCl and the precipitated protein removed by centrifugation at 3000*g* for 15 min. The supernatant, which contains glucosyl-galactosylhydroxylysine residue, was diluted to 8 ml with distilled water and the pH was adjusted to 2.0. This solution was applied to a 9 × 5-mm Dowex 50 column (H^+) and washed initially with 4 ml of 0.1 *M* citrate phosphate buffer, pH 3.0, followed by 50 ml of distilled water. The specific product, glucosyl-galactosylhydroxylysine, was eluted from the resin with 2.5 ml of 1.5 *M* NH_4OH, mixed with 10 ml of scintiverse (Fisher Scientific Co.) and counted in a liquid scintillation counter. Chromatographic determination of this eluate showed the presence of all radioactivity only in this fraction. Optimal conditions such as protein concentration, pH, and incubation time for enzyme activity were employed in the present study. The enzyme activity is expressed as dpm per milligram protein.

Determination of N-acetyl-β-glucosaminidase activity. Enzyme source was prepared as described above. The reaction mixture contained 200 μl of 0.5 *M* acetate buffer, pH 4.5, 100 μl of 0.012 *M* substrate (*P*-nitrophenyl-*N*-acetyl-D-glucosaminide), 10 μl of 5% Triton X-100, and μl of enzyme preparation. After an incubation for 1 hr at 37°C, the reaction was stopped by the addition of 2.5 ml of 0.1 *M* sodium carbonate, pH 8.0. After mixing thoroughly, the absorbance of the released *p*-nitrophenol was measured at 410 nm in a Beckman DB spectrophotometer. Standards of *p*-nitrophenol were run simultaneously with samples. The results are expressed as nanomoles of *p*-nitrophenol per milligram of glomerular protein per hour.

Protein determination. Protein in each supernatant was determined by the ultramicromethod using the Coomassie G 250 dye (Sedmak and Grossberg, 1977). Bovine serum albumin was used as a standard.

Determination of plasma sugars. Plasma sugars in all groups of rats were determined by the ferricyanide method of Hoffman (1937) in a Technicon autoanalyzer.

RESULTS

General information on some representative animals is set out in Table 1. Normal rats continued to grow until sacrifice, whereas growth in hypox rats was

TABLE I
General Information on Animals[a]

Rats	Body wt. (g)	Kidney wt. (g)	Plasma sugars (mg/dl)	Plasma[b] proline (μmole/ml)
Normal	343 ± 6 (19)	2.70 ± 0.05 (19)	135 ± 5 (13)	0.160 ± 0.014 (7)
Hypophysectomized	83 ± 2 (19)	0.54 ± 0.01 (19)	138 ± 7 (17)	0.075 ± 0.005 (8)**
Hypophysectomized + growth hormone	107 ± 3 (19)*	0.70 ± 0.02 (19)**	143 ± 5 (19)	0.168 ± 0.018 (4)
Diabetic	197 ± 10 (8)	2.36 ± 0.10 (8)	453 ± 27 (8)	0.142 ± 0.011 (4)
Diabetic + growth hormone	180 ± 15 (18)	2.13 ± 0.148 (18)	483 ± 30 (6)[b]	0.198 ± 0.016 (4)

[a] Values shown are means ± SEM. Numbers in parentheses indicate number of animals.

[b] Plasma pooled from two to three animals.

* Initial body wt 85 ± 2; hypophysectomized vs hypophysectomized + growth hormone: $P < 0.001$.

** Normal or hypophysectomized + growth hormone vs hypophysectomized: $P < 0.001$.

minimal. Treatment of hypox rats with GH resulted in 26% increase in body weight and 30% increase in kidney weight. Both increases were statistically significant. Plasma sugar values did not differ significantly among normal, hypox, and GH-treated hypox rats. Also, no differences in plasma sugar levels were found between diabetic and GH-treated diabetic rats. Plasma proline concentration was significantly lower in hypox rats than in normal rats. Administration of GH to hypox rats restored plasma proline concentrations to normal. No difference in plasma proline concentration was found between normal and diabetic or GH-treated diabetic rats.

The specific activity (SA) of proline in renal cortex fluid (tissue-free) in various groups of rats after a single injection of tritiated proline is shown in Table II. In all groups of rats, the SA of proline gradually declined with time. In hypox rats, the SA was found to be much higher than in normal rats, and it was restored to normal after GH treatment. The ratio of the area under the normal tissue-free proline SA curve to that under the hypox SA curve was found to be 1.47. This value was used to multiply the normal GBM proline and hydroxyproline specific activities. The ratio under the normal SA curve to that of GH-treated hypox rats

TABLE II
Tissue-Free Proline Specific Activity (DPM × 10^{-4}/μmole proline) in Normal, Hypophysectomized and Growth-Hormone-Treated Hypophysectomized Rats at Various Time Intervals after a Single Intraperitoneal Injection of L-[2,3,^{3}H]Proline

Time (hr)	Normal	Hypophysectomized	Hypophysectomized + growth hormone
1	9.77	9.44	5.47
2	6.00	7.89	5.08
4	4.08	6.78	4.49
12	3.47	4.94	3.79
24	2.11	3.80	3.05
36	2.02	3.29	2.65
48	1.63	3.07	2.23
84	0.87	2.86	1.83
120	0.26	1.44	0.87
188	0.21	1.09	0.45

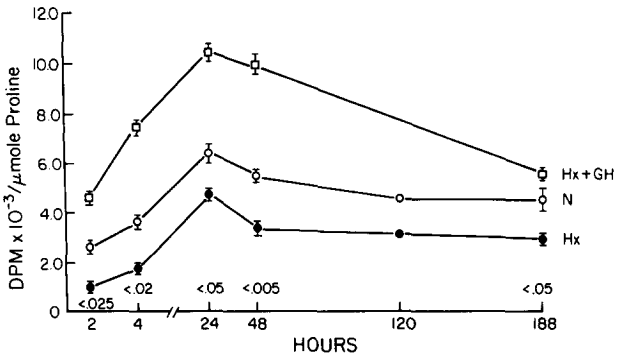

FIG. 1. Specific activity of proline of glomerular basement membrane at various times after the injection of L-[2,3,^{3}H]proline (140 μCi/100 g body wt) into normal (N), hypophysectomized (Hx), and growth-hormone-treated hypophysectomized (Hx + GH) rats. Each point represents the value of basement membranes isolated from the pooled kidney cortices of two to five rats. Number of observations at each point were two to three except at 120 hr. *P* values are between normal and hypophysectomized rats.

was 1.01. The ratio obtained between normal and diabetic rats was 1.70. A similar ratio was found between normal and GH-treated diabetic rats. No difference in tissue-free proline SA was obtained between normal and GH-treated normal rats or between saline-treated diabetic and GH-treated diabetic rats.

Figures 1 and 2 represent the incorporation of tritiated proline into proline and hydroxyproline of GBM of normal, hypox, and GH-treated hypox rats. In both normal and hypox rats, the specific activities of proline and hydroxyproline increased to maximum at 24 hr followed by a slight decline at 48 hr and thereafter remained relatively constant up to 188 hr. As evident, significant decreases in specific activities of both proline and hydroxyproline were found in hypox rats at all times of the study, when compared to normal rats. Treatment of hypox rats with GH restored the SA of hydroxyproline to normal, but there was an exacerated response of proline SA compared to normal rats.

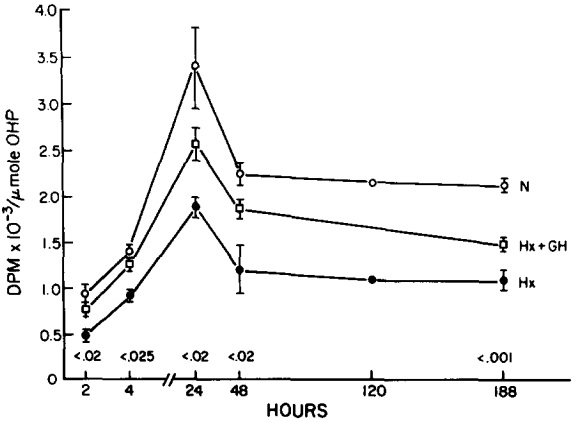

FIG. 2. Specific activity of hydroxyproline of glomerular basement membrane at various times after the injection of L-[2,3,^{3}H]proline (140 μCi/100 g body wt) into normal (N), hypophysectomized (Hx), and growth-hormone-treated hypophysectomized (Hx + GH) rats. Each point represents the value of basement membranes isolated from pooled kidney cortices of two to five rats. Number of observations at each point were two to three except at 120 hr. *P* values are between normal and hypophysectomized rats.

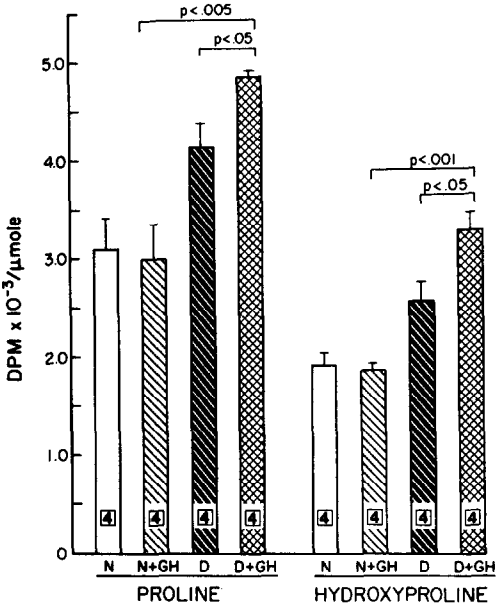

FIG. 3. Specific activities of proline and hydroxyproline of glomerular basement membrane 24 hr after the injection of L-[2,3,^{3}H]proline (140 μCi/100 g body wt) into normal (N), diabetic (D), growth-hormone-treated normal (N + GH), and growth-hormone-treated diabetic (D + GH) rats. Numbers in bars indicate number of observations. Each observation represents the value of basement membranes isolated from the pooled kidney cortices of two to four animals.

Figures 3 and 4 show the effect of diabetes and GH treatment in diabetic rats on GBM protein and collagen synthesis. These rats were sacrificed at 24 hr (Fig. 3) and 48 hr (Fig. 4) after the injection of [^{3}H]proline. As evident, both proline and hydroxyproline specific activities were higher in diabetic than in normal or GH-treated normal rats. Treatment of diabetic rats with GH resulted in significantly increased GBM protein and collagen synthesis when compared to diabetic rats.

Glucosyltransferase and *N*-acetyl-β-glucosaminidase activities in glomeruli from various groups of rats are shown in Table III. The glucosyltransferase activity was significantly lower in hypox and higher in diabetic rats than in normal rats. Treatment of hypox rats with GH restored the enzyme activity to normal. However, statistically nonsignificant increase in glucosyltransferase activity was found in GH-treated diabetic rats when compared to saline-treated diabetic rats. The activity of *N*-acetyl-β-glucosaminidase was significantly increased in glomeruli of hypox rats when compared to normal or GH-treated hypox rats.

DISCUSSION

The renal GBM is composed of collagenous and noncollagenous glycoprotein components (Spiro, 1976; Kefalides, 1978; Heathcote and Grant, 1981; Farquhar, 1982). The collagen component contains substantial amounts of glycine, proline, hydroxyproline, hydroxylysine, and half-cystine. Hydroxyproline is unique to collagen, and is formed by hydroxylation of proline subsequent to peptide linkage (Cardinale and Udenfriend, 1974). The rate of formation of hydroxyproline is regarded as a measure of total collagen synthesis. The noncollagenous component of the GBM contains proteoglycans, laminin, fibronectin, and entactin (Farquhar,

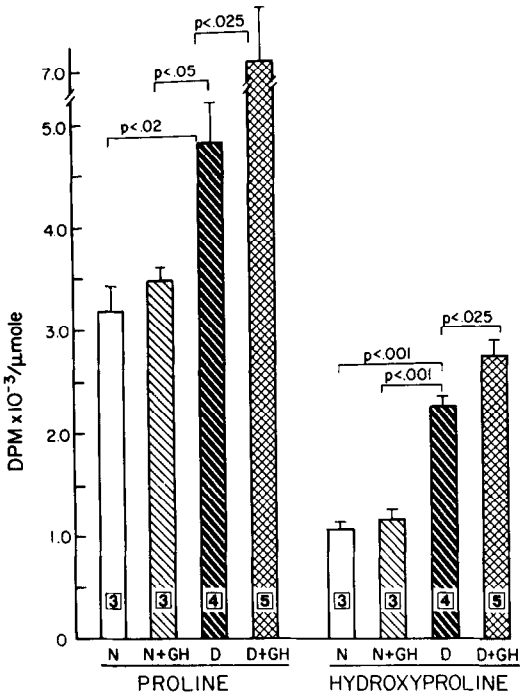

FIG. 4. Specific activities of proline and hydroxyproline of glomerular basement membrane 48 hr after the injection of L-[2,3,^{3}H]proline (140 μCi/100 g body wt) into normal (N), diabetic (D), growth-hormone-treated normal (N + GH), and growth-hormone-treated diabetic (D + GH) rats. Numbers in bars represent number of observations. Each observation was obtained from basement membranes isolated from the pooled kidney cortices of two to four animals.

1982). The predominant proteoglycan is heparan sulfate, which comprises about 85% of the total proteoglycans extracted from the GBM (Farquhar, 1982). Decreased content of heparan sulfate was reported in human diabetic GBM (Parthasarathy and Spiro, 1982) and kidney cortex of streptozotocin diabetic rats (Saraswati and Vasan, 1983). Also, decreased kidney proteoglycan synthesis has been reported in streptozotocin diabetic rats (Brown *et al.*, 1982; Kanwar *et al.*,

TABLE III
Glomerular Enzyme Activity in Various Groups of Rats[a]

Rats	No.	Glucosyltransferase (DPM × 10^{-3}/mg protein)	*N*-Acetyl-β-glucosaminidase (nmole *p*-nitrophenol/mg protein)
Normal	6	51.6 ± 3.6*	1625 ± 22***
Hypophysectomized	8	37.5 ± 3.9**	2539 ± 110†
Hypophysectomized + growth hormone	5	62.1 ± 3.6	1255 ± 182
Diabetic	4	68.6 ± 4.4	—
Diabetic + growth hormone	4	73.9 ± 2.4	—

[a] Values shown are means ± SEM.
* Normal vs hypophysectomized: $P < 0.05$; normal vs diabetic: $P < 0.02$.
** Hypophysectomized vs hypophysectomized + growth hormone: $P < 0.001$.
*** Normal vs hypophysectomized: $P < 0.001$.
† Hypophysectomized vs hypophysectomized + growth hormone: $P < 0.001$.

1983; Cohen and Surma, 1984) and in EHS tumor grown in genetically diabetic mice (Rohrbach *et al.*, 1982). Since heparan sulfate carries a net negative charge on the GBM, it is likely that a decrease in synthesis and concentration of heparan sulfate results in altered electrostatic properties of the GBM and thus causes proteinuria (Kanwar, 1984). The influence of proteoglycans and other noncollagenous glycoproteins on collagen metabolism either in diabetes or other kidney diseases is not clearly understood at the present time.

Although changes in the chemistry and metabolism of diabetic GBM have been reported, the pathogenesis of diabetic microangiopathy remains unclear. Hyperglycemia and/or insulin deficiency have been implicated as major causes of chronic complications of diabetes in both animal and human studies. Although other metabolic derangements have been suggested, no direct supportive evidence is available. Lundbaek and associates (Lundbaek *et al.*, 1970, 1980) have proposed GH as a causal factor in the development of diabetic angiopathy. Pituitary ablation has not only proven beneficial in improving diabetic retinopathy, but also resulted in the reduction of GBM thickness (four out of five patients) and in the restoration of previously atrophic endothelial and mesangial cells (Ireland *et al.*, 1967). Koletsky and Snajdar (1979) demonstrated diminished proteinuria in genetically obese rats following hypophysectomy. Ooshima *et al.* (1977) reported significantly decreased prolyl hydroxylase activity and collagen content in the aorta of hypox rats and partial restoration toward normal after GH treatment for 2 weeks. Meyer *et al.* (1983) reported that pituitary ablation caused significant reductions in single nephron glomerular filtration rate, mean transcapillary hydraulic pressure difference, and proteinuria in adult rats with 90% surgical removal of nephron mass compared to those in sham-operated rats. However, the role of hypophysectomy in GBM collagen synthesis has not been studied either in normal or diabetic rats. The results of the present study demonstrate that collagen synthesis is significantly decreased in the GBM of hypox rats and increased in diabetic rats. Administration of GH to hypox rats restored the synthesis of GBM collagen to normal. Also, GH treatment in diabetic rats caused a significant increase in both GBM collagen and protein synthesis when compared to saline-treated diabetic rats, suggesting the involvement of GH in the metabolism of GBM collagen. These data provide direct biochemical evidence in support of GH hypothesis in the pathogenesis of diabetic microvascular complications, as proposed by Lundbaek and associates (Lundbaek *et al.*, 1970, 1980).

A recent report by Rabin *et al.* (1984) suggested that a patient with GH deficiency and insulin-requiring diabetes for 24 years following pancreatectomy developed background retinopathy, peripheral neuropathy, and nephropathy with proteinuria. This report questions the possible role of GH in the development of long-term complications of diabetes. Although these results need further confirmation, the possibility that fluctuating hyperglycemia over the period of 24 years was responsible for these complications and that the absence of GH simply delayed the progression of the complications to total blindness and end stage renal failure cannot be excluded.

In addition of collagen synthesis, GH seems to have profound influence on enzymes involved in collagen and noncollagenous glycoprotein metabolism. Glucosyltransferase is an enzyme involved in biosynthesis of the disaccharide unit of basement membranes and collagen, and the activity of this enzyme has been shown to reflect the rate of basement membrane synthesis (Spiro and Spiro,

1971a). Elevated levels of glucosyltransferase were reported in renal cortical homogenates of diabetic animals (Spiro and Spiro, 1971b; Grant *et al.*, 1976; Haft and Reddi, 1979; Chang *et al.*, 1980; Schmidt *et al.*, 1980; Bretzel *et al.*, 1981; Heathcote *et al.*, 1981). In contrast, the activity of glucosyltransferase was significantly lower in glomeruli of hypox than in normal rats. GH treatment restored the enzyme activity to normal. Diabetic glomeruli had significantly higher glucosyltransferase activity than normal glomeruli. Although GH increased the enzyme activity by 8% in diabetic rats, this effect was not statistically significant. These data suggest that GH influences kidney glucosyltransferase and the effect is opposite to that of insulin, which was found to decrease the elevated enzyme activity in diabetic rats to normal (Spiro and Spiro, 1971b).

Enzymes involved in the degradation of glycoprotein such as α-glucosidase, β-galactosidase and N-acetyl-β-glucosaminidase were found to be decreased in renal cortical homogenates of diabetic rats (Fushimi and Tarui, 1974; 1976a, b). In hypox rats, the activity of *N*-acetyl-β-glucosaminidase was significantly increased. These results are in contrast to those observed in diabetic rats. Normalization of enzyme activity in GH-treated hypox rats suggests that GH influences noncollagen glycoprotein metabolism by altering glycosidase activity.

It should be noted that hypox rats are devoid of many anterior pituitary hormones. In view of this, the decreased GBM collagen synthesis in hypox rats is difficult to interpret. However, the reversal of GBM collagen synthesis by GH administration suggests that the metabolism of GBM is influenced by GH. Furthermore, GH treatment caused enhanced skin and femur collagen synthesis in normal rats (Aer *et al.*, 1968). The possible roles of thyroxine, adrenal steroids, testosterone, prolactin, etc. in GBM metabolism remain to be studied.

GH seems to enhance protein synthesis, hydroxylation, and glycosylation reactions in the biosynthesis of GBM collagen. Although no data are available at the present time, it is possible that GH may decrease the breakdown of GBM collagen. These events may result in enhanced deposition of GBM collagen in conditions that are associated with GH excess. Since diminished proteinuria was observed in rats following hypophysectomy (Koletsky and Snajdar, 1979; Meyer *et al.*, 1983), it is possible that GH may also influence the metabolism of proteoglycans and other noncollagenous glycoprotein components of the GBM.

It is apparent from Table II that the SA of proline was substantially lower in normal than in hypox rats. Consequently, a valid comparison of GBM collagen synthesis *in vivo* between normal and hypox rats could not be made without correcting the differences in the SA of the immediate precursor pool, which is the tissue-free proline SA. Studies of Fern and Garlick (1974) have shown that tissue-free amino acid pools represent the more immediate precursors used for protein synthesis rather than plasma amino acid pool. Although the reason for higher tissue-free proline SA in hypox rats is not known, it is possible that in these rats the injected tracer may be less diluted by the available proline pool or there may be less peripheral output of unlabeled proline from protein breakdown for intra- or extracellular dilution.

In summary, the results of the present study suggest that the synthesis of GBM collagen is decreased in hypox rats, and administration of GH to these rats restores GBM collagen synthesis to normal. Also, treatment of diabetic rats with GH increases further the synthesis of GBM collagen. The role of GH in GBM

metabolism may be of particular significance in view of its involvement in diabetic microsvascular complications.

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