Effect of Insulin on High-Glucose Medium-Induced Changes in Rat Glomerular Epithelial Cell Metabolism of Glycoconjugates

B. S. Kasinath

With the technical assistance of William C. Terhune and Rolando Maldonado

Department of Medicine, University of Texas Health Science Center, Audie L. Murphy Memorial Veterans' Administration Hospital, San Antonio, Texas 78284

Received August 15, 1994, and in revised form February 3, 1995

We have previously reported that incubation of rat glomerular epithelial cells in vitro for 8 days with 30 mm glucose without insulin results in reduction in the synthesis of a cell layer heparan sulfate proteoglycan (HSPG) species that has hydrodynamic size and antigenic characteristics of glomerular basement membrane HSPG (1994, Arch. Biochem. Biophys 309, 149-159). In these studies, reduction in HSPG synthesis could be attributed either to high-glucose medium or to insulin deficiency. In this study we investigated the effects of insulin replacement on changes in glomerular epithelial cell metabolism of glycoconjugates induced by high-glucose medium. Addition of pharmacologic concentrations of insulin prevented the following changes induced by 30 mm glucose: (a) increment in $^{35}S$O$_4$ incorporation into macromolecules in cell layer and medium, (b) increment in the synthesis of low anionic macromolecules, probably glycoproteins, in both cell layer and medium, (c) increment in synthesis of small-sized glycosaminoglycans ($K_r$, 0.75 on Sephrose CL-4B) associated with the cell layer. Insulin was unable to correct the 30 mm glucose-induced reduction in the synthesis of cell layer HSPG that resembles glomerular basement membrane HSPG. Physiologic concentrations of insulin did not affect any of the changes in glycopeptide metabolism induced by 30 mm glucose. These findings suggest that (a) inhibition of glomerular epithelial cell synthesis of $^{35}S$O$_4$-labeled low anionic macromolecules, probably glycoproteins, may be involved in insulin-induced reversal of glomerular hypertrophy seen in early diabetes, and (b) mechanisms other than insulin lack are involved in the synthesis of glomerular basement membrane HSPG in diabetic nephropathy.

Key Words: proteoglycans; diabetic nephropathy; basement membrane; insulin.

Glomerular involvement in diabetic renal disease is characterized by changes in both morphology and function. Morphological alterations include thickening of glomerular basement membrane (1), reduction in anionic site density (2), and expansion of mesangial matrix and increase in fractional mesangial volume (3). Functional changes at the glomerular level include initial increment in the rate of glomerular filtration which declines with passage of time and impairment in the selective permeability function, resulting in proteinuria (1). The pathogenesis of proteinuria is thought to be multifactorial, involving hemodynamic (4) and biochemical mechanisms (5). The biochemical mechanisms involve changes in the constituents of the glomerular basement membrane including proteoglycans (6, 7).

Recognition of proteoglycans, especially heparan sulfate proteoglycan (HSPG)$^2$ as important regulators of both charge- and size-selective aspects of glomerular permselectivity (8, 9) has led to investigation of HSPG metabolism in diabetic renal disease. Studies in diabetic rats have suggested several abnormalities in glomerular proteoglycan metabolism including reduction

---

$^1$To whom correspondence should be addressed at Division of Nephrology, Department of Medicine, University of Texas, Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284. Fax: (210) 567-4712.

$^2$Abbreviations used: Chaps, 3-[3-cholamidopropyl]dimethylammonio)propanesulfonate; CS/DSPG, chondroitin sulfate/dermatan sulfate proteoglycan; FBS, fetal bovine serum; GAG, glycosaminoglycan; GEC glomerular epithelial cells; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HSPG, heparan sulfate proteoglycan; PBS, phosphate-buffered saline.
in the synthesis of glomerular basement membrane HSPG (5), undersulfation (10), and molecular abnormalities resulting in inefficient binding to other matrix components (11). Recently, we have addressed the cellular basis of derangements in HSPG content of the diabetic glomerular basement membrane. Our investigations have shown that the rate of synthesis of a cell layer-associated HSPG species that resembles glomerular basement membrane HSPG in hydrodynamic size and antigenic characteristics is reduced when glomerular epithelial cells are exposed to high-glucose (30 mM) medium in the absence of insulin (12).

The mechanisms by which the environment of hyperglycemia and insulin lack results in glomerular HSPG dysregulation are not known. As type 1 diabetes is characterized by insulin deficiency, the lack of this hormone could conceivably contribute to disturbances in HSPG metabolism in diabetes. In the present study, we examined whether insulin can prevent reduction in glomerular basement membrane HSPG synthesis and rectify other changes in glycopeptide metabolism of the glomerular epithelial cells induced by 30 mM glucose.

**METHODS**

**Cell cultures.** Glomerular epithelial cells (GEC) of the rat (kindly provided by Dr. Jeffrey I. Kreisberg, University of Texas Health Science Center, San Antonio) were grown in culture and characterized as described by us previously (12–15). GEC in vitro display several phenotypic characteristics of GEC in vivo, including expression of glomerular basement membrane HSPG (12, 13), antigen of Heymann nephritis (15), type IV collagen, and entactin (unpublished observations). GEC were grown to confluence in 12-well dishes in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 15 mM Hepes, insulin, and antibiotics. On the day of the experiment the medium was changed to: (a) RPMI containing 5 mM glucose, 0.66 unit/ml insulin, 10% FBS, and additives as above, (b) RPMI containing 30 mM glucose, 10% FBS, no added insulin, and additives as above, or (c) RPMI containing 30 mM glucose, 10% FBS, 0.66 unit/ml insulin and additives as above. Whereas majority of experiments were performed employing pharmacologic concentrations of insulin routinely employed in cell culture, the effects of physiologic concentrations of insulin (15 µU/ml) (16) were also studied in separate experiments. The media were changed on Days 3 and 7 with media of respective composition. On Day 7 the cells were labeled for 24 h with 200 pCi/ml of carrier-free [35 S]SO4 (ICN radiochemicals, sp act 43 pCi/mg 5). The data (CPM per mg cell protein) in each condition was expressed as 0.05 was considered significant.

**Isolation of labeled macromolecules.** Labeled macromolecules were separated from low anionic labeled macromolecules by DEAE-Sepharose chromatography by previously published methods (12, 17). Equal amounts of pooled 35S-labeled macromolecules from each incubation condition were applied to a 1.0- to 2.0-ml bed volume DEAE-Sepharose column and eluted in 1-ml fractions along a linear NaCl gradient employing 6 M urea buffer (see above) containing 0.15 M NaCl and 1.5 M NaCl as start and limit buffers, respectively. Protease inhibitors were included in both buffers. Gradient development was monitored by measurement of conductivity of fractions using CDM-80 instrument (Radiometer, Copenhagen, Denmark). The fractions eluting at greater than 0.5 M NaCl were pooled as proteoglycan (12, 17).

**Molecular sieve chromatography.** The hydrodynamic size characteristics of proteoglycans were studied by Sepharose CL-4B chromatography as previously described (12, 17). Equal amounts of labeled proteoglycans from each condition of incubation were eluted in 1-m1 fractions from a 100 x 0.65-cm column of Sepharose CL-4B under dissociative conditions employing 4 M guanidine–HCl, 50 mM Tris–HCl, 0.5% Triton X-100, and protease inhibitors. Aliquots of fractions were counted and fractions corresponding to the peaks were pooled. The hydrodynamic size of proteoglycans was calculated as Rm = V/S x, where Vm is the void volume determined by blue dextran elution, V is the total volume determined by elution of free (‘H)–glucosamine, and, x, is the elution volume of labeled proteoglycan.

**Glycosaminoglycan (GAG) digestion procedures.** These were performed as previously described by us (12, 17). Proteoglycans in each peak of Sepharose CL-4B chromatography were concentrated and exchanged into water by microconcentrators. Each pool was then divided into three aliquots and subjected to chondroitinase-ABC or nitrous acid digestion; the third untreated aliquot served as a control. Following GAG digestion gel chromatography was performed using a G-50 Sephadex column with PBS as the elution buffer and relative proportions of chondroitin sulfate and heparan sulfate proteoglycans were determined.

**Immunoprecipitation.** Confluent layers of GEC were incubated with medium containing 5 mM glucose and insulin, 30 mM glucose without added insulin, or 30 mM glucose with insulin for 7 days and labeled with [35 S]SO4 (200 µCi/ml) for 24 h as described above. The cell layers were washed in respective medium and cell layers dissolved in 1% NP-40 buffer. The protein content of the cell lysates was estimated by a commercially available kit (Biorad) and the protein A-Sepharose at 4°C for 60 min with constant agitation. The beads were washed three times in a buffer containing 150 mM NaCl, 20 mM Tris–HCl, pH 7.4, 1% NP-40, 1% Triton X-100, 0.05% SDS (buffer A) and incubated with cell lysate for 2 h at 4°C with constant agitation. The immunoprecipitates were washed sequentially six times in buffer A, three times 0.5 M lithium chloride in 0.1 M Tris–HCl, pH 7.5, and once each in buffer A and 50 mM Tris HCl, pH 7.4. The immunoprecipitates were then counted using a beta counter. The nonspecific cpm of preimmune serum were subtracted from cpm of antibody binding to derive net specific cpm of immunoprecipitates.

**Statistics.** The data (CPM per mg cell protein) in each condition of incubation from three to seven experiments were expressed as mean ± SE and compared by Student’s t test; P < 0.05 was considered to be statistically significant.

**RESULTS**

**Studies on 35S Incorporation into Macromolecules**

The GEC incorporated the label into macromolecules that were distributed into cell layer and medium com-
In the cell layer, 30 mM glucose induced a statistically significant 1.6-fold increase in the synthesis of $^{35}$S-labeled low-anionic macromolecules and 1.7-fold increment in the synthesis of proteoglycans; both these parameters were restored to control levels by insulin (Table I).

In the medium, 30 mM glucose induced a significant fourfold rise in the synthesis of $^{35}$S-labeled low-anionic macromolecules (Table II). This was prevented by the addition of insulin. Although nearly 23% increment in synthesis of medium proteoglycans was seen following incubation with 30 mM glucose, this change did not reach statistical significance; insulin decreased the rate of medium proteoglycan synthesis to control levels (Table II).

### Insulin Effects on High-Glucose Medium-Induced Changes in Proteoglycan Metabolism

#### Cell layer proteoglycans

Control cell layer proteoglycans resolved into two peaks of $K_a$ 0.4 and 0.75 on dissociative Sepharose CL-4B chromatography ac-

#### TABLE I

<table>
<thead>
<tr>
<th></th>
<th>5 mM Glucose + insulin</th>
<th>30 mM Glucose</th>
<th>30 mM Glucose + insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{35}$SO$_4$-labeled macromolecules</td>
<td>Ion-exchange chromatography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak A</td>
<td>18 ± 1.7</td>
<td>29 ± 3.6*</td>
<td>20 ± 5.4</td>
</tr>
<tr>
<td>Peak B</td>
<td>82 ± 1.7</td>
<td>140 ± 16.4***</td>
<td>79 ± 11.7†</td>
</tr>
<tr>
<td>(Proteoglycans)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepharose CL-4B chromatography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak I</td>
<td>31 ± 2.6</td>
<td>21 ± 3.5*</td>
<td>23 ± 6.5</td>
</tr>
<tr>
<td>HS</td>
<td>24 ± 2.4</td>
<td>16.5 ± 3.3**</td>
<td>15.3 ± 3.8**</td>
</tr>
<tr>
<td>CS/DS</td>
<td>7 ± 0.9</td>
<td>4.5 ± 1.1</td>
<td>7.7 ± 2.7</td>
</tr>
<tr>
<td>Peak II</td>
<td>51 ± 2.4</td>
<td>119 ± 12.8***</td>
<td>56 ± 5.2‡</td>
</tr>
<tr>
<td>HS</td>
<td>38 ± 2.9</td>
<td>112 ± 12.5***</td>
<td>48 ± 19*</td>
</tr>
<tr>
<td>CS/DS</td>
<td>12 ± 1.7</td>
<td>7 ± 2.0*</td>
<td>8 ± 3.5</td>
</tr>
</tbody>
</table>

*Note.* The incorporation of $^{35}$SO$_4$ into macromolecules in control (5 mM glucose + insulin) cell layer from different experiments was normalized to the figure shown. The data for 30 mM glucose without insulin and with added insulin were then calculated according to percentage difference in label incorporation from control in each experiment. The figures represent pooled data from seven experiments each for 5 mM glucose and 30 mM glucose without added insulin and three experiments for 30 mM glucose with insulin. HS, heparan sulfate; CS, chondroitin sulfate.

* $P < 0.025$ compared to 5 mM glucose with insulin.
** $P < 0.05$ compared to 5 mM glucose with insulin.
*** $P < 0.001$ compared to 5 mM glucose with insulin.
† $P < 0.01$ compared to 30 mM glucose.

#### TABLE II

<table>
<thead>
<tr>
<th></th>
<th>5 mM Glucose + insulin</th>
<th>30 mM Glucose</th>
<th>30 mM Glucose + insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{35}$SO$_4$-labeled macromolecules</td>
<td>Ion exchange chromatography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak A</td>
<td>5 ± 0.9</td>
<td>20 ± 1.6*</td>
<td>8 ± 2.9†</td>
</tr>
<tr>
<td>Peak B</td>
<td>22 ± 1.6</td>
<td>27 ± 3.6</td>
<td>20 ± 3.8</td>
</tr>
<tr>
<td>(proteoglycans)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepharose CL-4B chromatography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak I</td>
<td>18 ± 1.5</td>
<td>20 ± 3.0</td>
<td>12 ± 0.9‡***</td>
</tr>
<tr>
<td>HS</td>
<td>15 ± 1.4</td>
<td>15.6 ± 1.6</td>
<td>10 ± 1.2***</td>
</tr>
<tr>
<td>CS/DS</td>
<td>3 ± 0.3</td>
<td>4.4 ± 1.7</td>
<td>2 ± 0.3</td>
</tr>
<tr>
<td>Peak II</td>
<td>4 ± 0.7</td>
<td>7 ± 1.8</td>
<td>8 ± 2.9</td>
</tr>
<tr>
<td>HS</td>
<td>1.5 ± 0.3</td>
<td>3 ± 0.8</td>
<td>4.5 ± 1.6</td>
</tr>
<tr>
<td>CS/DS</td>
<td>2.5 ± 0.5</td>
<td>4 ± 1.3</td>
<td>3.5 ± 1.4</td>
</tr>
</tbody>
</table>

*Note.* The incorporation of $^{35}$SO$_4$ into macromolecules in the medium of cells grown in medium containing 5 mM glucose + insulin (control) from different experiments was normalized to the figure shown maintaining the proportion relative to the incorporation of the label into macromolecules in cell layer. The data for 30 mM glucose without added insulin and with added insulin were then calculated according to percentage difference in label incorporation from control in each experiment. The figures represent pooled data from seven experiments each for 5 mM glucose and 30 mM glucose without added insulin and three experiments for 30 mM glucose with insulin. HS, heparan sulfate; CS, chondroitin sulfate.

* $P < 0.001$ compared to 5 mM glucose + insulin.
** $P < 0.01$ compared to 5 mM glucose + insulin.
*** $P < 0.05$ compared to 5 mM glucose + insulin.
† $P < 0.01$ compared to 30 mM glucose.
‡ $P < 0.05$ compared to 30 mM glucose.

#### Changes in Proteoglycan Metabolism

As previously reported by us, analysis of $^{35}$S-labeled macromolecules in cell layer and medium by DEAE-Sephael chromatography yields two prominent peaks (12). The first peak eluting at 0.15–0.18 M NaCl consists of low anionic, $^{35}$S-labeled macromolecules, probably glycoproteins, whereas the second peak eluting at >0.3 M NaCl is composed of proteoglycans (Figs. 1 and 2). An additional small peak eluting at approximately 0.2 M NaCl was also seen in medium, but could not be analyzed due to small amounts available. Proteoglycans (peaks B) in medium eluted at similar concentrations of NaCl in the three groups; a similar pattern was seen with cell layer proteoglycans (Figs. 1 and 2). These data suggest that incubation with 30 mM glucose with or without insulin did not alter the overall anionic charge density of medium and cell layer proteoglycans.
INSULIN EFFECTS ON PROTEOGLYCANS

FIG. 1. Ion exchange chromatography of $^{35}$SO$_4$-labeled cell layer-associated macromolecules from cells incubated with (A) 5 mM glucose with insulin, (B) 30 mM glucose without added insulin, and (C) 30 mM glucose with insulin. Equal amounts (cpm) of labeled material from the three conditions of incubation were subjected to DEAE-Sephacel chromatography. Fractions containing proteoglycans in peaks B were pooled as indicated by the bars.

FIG. 2. Ion exchange chromatography of $^{35}$SO$_4$-labeled medium macromolecules from cells incubated with (A) 5 mM glucose with insulin, (B) 30 mM glucose without added insulin, and (C) 30 mM glucose with insulin. Equal amounts (cpm) of labeled material from the three conditions of incubation were subjected to DEAE-Sephacel chromatography. Fractions containing proteoglycans in peaks B were pooled as indicated by the bars.

drodynamic size of glomerular basement membrane HSPG (12, 20) and could be immunoprecipitated with antibody against rat glomerular basement membrane HSPG (12).

Glucose (30 mM) induced a significant increase (70%) in the synthesis of cell layer associated proteoglycans which was completely abolished by insulin replacement (Fig. 4A; Table I). The effect of 30 mM glucose on cell layer proteoglycans present in the individual peaks counting for 38 and 62% of all cell layer proteoglycans, respectively (Fig. 3A), as previously reported (12). Specific digestion procedures showed that heparan sulfate accounted for nearly 77% of both peaks (Table I). Studies employing alkaline hydrolysis had demonstrated that peak C-I consisted of intact proteoglycans, whereas peak C-II was constituted by free GAG chains (12). HSPG in peak C-I ($K_a$, 0.4) approximates the hy-
was distinctive. Glucose (30 mM) induced a significant reduction in the synthesis of peak C-I proteoglycans (31,000 ± 2600 vs 21,000 ± 3500 cpm/mg cell protein, \( P < 0.025 \)) (Fig. 3; Table I); insulin was not able to rectify this decrease and restore the rate of synthesis to control levels (Fig. 3; Table I). GAG analysis showed that synthesis of HSPG species in peak C-I which resembles glomerular basement membrane HSPG was also significantly decreased (24,000 ± 2400 vs 16,500 ± 3300 cpm/mg cell protein, \( P < 0.05 \)) (Fig. 4B; Table I); addition of insulin to 30 mM glucose failed to restore the synthesis of this species to normal (Fig. 4B; Table I).

Further confirmation was obtained by immunoprecipitation employing a specific antibody against GBM HSPG (Table III). Incubation of GEC with 30 mM glucose caused approximately 60% reduction (mean of two experiments) in the synthesis of \(^{35}\)SO₄-labeled glomerular basement membrane HSPG; addition of insulin did not prevent this reduction. The changes in the synthesis of CSPG in peak C-I induced by 30 mM glucose without or with added insulin did not reach statistical significance compared to control (Table I).

In contrast to reduction in synthesis of peak C-I proteoglycans, 30 mM glucose induced nearly a twofold increment in the synthesis of peak C-II GAGs, including that of heparan sulfate (Table I). Insulin completely prevented the increased synthesis of peak C-II GAGs, including that of heparan sulfate (112,000 ± 12,500 vs

![FIG. 3. Sepharose CL-4B chromatography of cell layer proteoglycans eluted with 4 M guanidine-HCl dissociative buffer containing protease inhibitors. Bars indicate pooling of fractions corresponding to peaks. (A) 5 mM Glucose with insulin, (B) 30 mM glucose without added insulin, (C) 30 mM glucose with insulin. Peaks C-I in B and C were pooled taking into consideration elution of peak C-I in A and geometry of elution of peaks C-II in B and C. Subsequent elution of peak C-I in B, and C on Sepharose CL-4B column showed the presence of proteoglycans of \( K_w \), 0.4, similar to control peak C-I in A.]

![FIG. 4. Effect of 5 mM glucose with insulin (5mMG), 30 mM glucose without added insulin (30mMG), and 30 mM glucose with insulin (30mMG + I) on the synthesis of \(^{35}\)S-labeled proteoglycans. (A) Total cell layer-associated proteoglycans. (B) Peak C-I HSPG that resembles glomerular basement membrane HSPG. *\( P < 0.05 \), **\( P < 0.001 \) compared to 5 mM glucose; #\( P < 0.01 \) compared to 30 mM glucose.]}
TABLE III

<table>
<thead>
<tr>
<th></th>
<th>5 mM Glucose + insulin</th>
<th>30 mM Glucose</th>
<th>30 mM Glucose + insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td>341</td>
<td>173</td>
<td>68</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>258</td>
<td>76</td>
<td>39</td>
</tr>
</tbody>
</table>

Note. GEC were incubated for 7 days with media containing 5 mM glucose + insulin and 30 mM glucose without and with added insulin and labeled with 200 μCi/ml of 35SO4 for 24 h. Equal amounts of cell lysate protein from each condition of incubation were employed in immunoprecipitation with antibody against rat glomerular basement membrane HSPG core protein (please see text for details). Data of net 35S cpm of immunoprecipitates from two experiments are shown.

48,000 ± 1900, P < 0.001) (Table I). Insulin, however, did not affect reduction in synthesis of peak C-II chondroitin sulfate induced by 30 mM glucose (Table I).

Medium proteoglycans. Control (5 mM glucose + insulin) medium proteoglycans also resolved into two peaks of $K_a, 0.14$ and 0.6 on Sepharose CL-4B chromatography as previously reported (12). Peak M-I accounted for nearly 82% of total proteoglycans in the medium. Incubation with 30 mM glucose did not significantly alter the rate of synthesis or the hydrodynamic size of constituent peaks (Fig. 5). Addition of insulin to 30 mM glucose resulted in reduction in synthesis of peak M-I proteoglycans ($P < 0.05$) (Table II). Peak M-I of control cells was composed mainly of HSPG and reduction in synthesis of peak M-I proteoglycans induced by insulin was accounted for mostly by decrease in synthesis of HSPG (Table II). Synthesis of peak M-II proteoglycans ($K_a, 0.6$) was unaffected by either incubation with 30 mM glucose or by addition of insulin to 30 mM glucose. Insulin did not alter the hydrodynamic sizes of medium proteoglycans.

Addition of physiologic concentrations of insulin (15 μU/ml) did not affect any of the above-described changes in 35S-labeled glycopeptide metabolism induced by 30 mM glucose. Furthermore, 35SO4 incorporation into macromolecules by cells incubated in 5 mM glucose was unaffected by removal of insulin.

DISCUSSION

Our studies demonstrate that insulin, at pharmacologic concentrations, was able to prevent the following changes in metabolism of 35S-labeled glycopeptides induced by 30 mM glucose: (i) increment in 35SO4 incorporation into macromolecules in cell layer and medium, (ii) increment in synthesis of 35S-labeled low-anionic macromolecules, probably glycoproteins, in cell layer and medium, (iii) increment in synthesis of a small-sized cell layer HS glycosaminoglycan chains ($K_a, 0.75$). Insulin, however, failed to prevent 30 mM glucose induced reduction in synthesis of HSPG associated with the cell layer that resembles glomerular basement membrane HSPG.

Following the detection of proteoglycans, particularly HSPG, as major determinants of anionic sites in the glomerular basement membrane (21), and demonstration that they regulate both charge- and size-selective aspects of glomerular permeability (8, 9) the status of these polyanionic macromolecules has been examined in a number of proteinuric disorders including diabetic nephropathy (6, 7). Reduction in absolute HSPG...
content of the glomerular basement membrane has been shown in both experimental and human diabetic nephropathy (22, 23). A reduction in the synthesis of glomerular basement membrane HSPG has been suggested as an underlying mechanism of proteinuria in diabetic nephropathy (5), although additional potential mechanisms such as inability to form effective molecular network with other basement membrane constituents have also been raised (11).

As GEC have been identified as the cellular source of glomerular basement membrane HSPG (24), it is conceivable that abnormalities in HSPG metabolism of the GEC results in the reduction in the glomerular basement membrane content of the HSPG. This hypothesis has been supported by our recent observations (12). When GEC in vitro were incubated with 30 mM glucose without added insulin for 8 days, there was a significant reduction in the synthesis of cell layer-associated HSPG which resembles glomerular basement membrane HSPG in hydrodynamic size and antigenic characteristics. This effect was not seen with equimolar mannitol employed as osmotic control. Although an osmotic mechanism was excluded, whether this reduction in HSPG synthesis was due to lack of insulin or due to some other metabolic consequence of exposure to high-glucose medium was unclear in our previous studies.

In the present study insulin significantly reduced the increment in the synthesis of 35S-labeled macromolecules induced by 30 mM glucose. Analysis by ion exchange chromatography showed that the high-medium glucose induced an increment in the synthesis of 35S-labeled glycoproteins in medium and cell layer, and cell layer-associated proteoglycans, although increase in the synthesis of medium proteoglycans did not achieve statistical significance. The high-glucose-induced changes in metabolism of cell layer peak II GAGs are of interest. Our previous observations suggest that high-glucose-induced increment in peak C II GAGs is due to enhanced synthesis (12). Additionally, increase in peak C II GAGs could also be due to increased rate of breakdown of proteoglycans. Brown et al. have reported augmented catabolism of glomerular proteoglycans in diabetes (25). The increase in peak C-II GAGs could be completely reversed by insulin. Although the mechanisms involved in insulin-mediated reversal are unknown, they may involve regulation of enzymes involved in metabolism of proteoglycans.

Increase in synthesis by high glucose of 35S-labeled glycoproteins correlates with the increment in synthesis of glomerular basement membrane sulfated glycoproteins reported in streptozotocin-induced diabetes (11). The increment in glycoprotein synthesis may contribute to the increase in the glomerular basement membrane matrix and hypertrophy of the glomeruli which occur early in diabetes and contribute to the renal hypertrophy (26–29). Following administration of pharmacologic doses of insulin to diabetic rats in order to achieve normoglycemia, reversal of both glomerular hypertrophy and thickness of the glomerular basement membrane to control levels was observed (28). The cellular basis for these observations has been unexplored until now. Our data suggest that reversal of glomerular hypertrophy induced by insulin may be partly mediated by a reduction in the synthesis of 35S-labeled glomerular glycoproteins. The sulfated glycoproteins in the glomerular basement membrane have not been studied in great detail. Enactin (nidogen) is a sulfated glycoprotein widely distributed in extracellular matrices and its content is increased in diabetic glomerular basement membrane (30). Studies on parietal endodermal cells that synthesize Reichert's membrane have shown that laminin and fibronectin are also sulfated (31). The status of laminin in diabetic glomerulus is controversial, with both decrease (23) and increase (32) being reported. Similarly, glomerular fibronectin content has been reported to be either normal (23) or increased (32). In addition, several other sulfated glycoproteins in the range of 50 to 180 kDa have been identified in glomerular basement membrane (33, 34) and Reichert's membrane (31, 35, 36). These sulfated glycoproteins await further characterization and their fate in diabetic nephropathy is unknown.

Insulin modulation of proteoglycans in diabetic renal tissue has not been studied extensively. Unger et al. reported that reduction in CSPG synthesis seen in articular cartilage in diabetic rats could be completely restored by administration of pharmacologic doses of insulin (37). In EHS tumor implanted into diabetic mice the basement membrane HSPG synthesis was reduced and was restored by administration of large doses of insulin (38). When data from previous studies and our present investigation are considered it appears that insulin modulation of hyperglycemia-induced alterations in proteoglycan metabolism is both tissue/cell-specific and specific for individual species of proteoglycans. In our study insulin restored high-glucose-induced stimulation of synthesis of a variety of medium and cell layer proteoglycans and GAGs to control levels; however, it was unable to rectify the reduction in GEC synthesis of cell-associated HSPG that resembles glomerular basement membrane HSPG.

Our data exclude insulin lack as a mechanism and implicate high glucose in the reduction of synthesis of glomerular basement membrane HSPG in diabetic nephropathy. The potential mechanisms could include disturbances in polyol metabolism (39), nonenzymatic glycation (40), and growth factors such as TGF-β (41). Derangements in polyol metabolism consisting of elevation in intracellular sorbitol and/or decrease in cellular myo-inositol have been linked to changes in glomerular filtration function and proteinuria in diabetes. Ad-
ministration of three structurally dissimilar inhibitors of aldose reductase which corrects elevated sorbitol content, was found to reduce urinary albumin losses in diabetic rates (42). Amelioration of urinary losses of albumin and IgG was also found when diabetic rats were fed a diet supplemented with myo-inositol in an effort to rectify cellular deficiency of this polyol (43). Although beneficial effects of these maneuvers on proteinuria suggest a link between polyol derangements and glomerular metabolism of HSPG, this issue has not been directly studied. Recently, TGFβ has been implicated in changes in metabolism of extracellular matrix components in diabetic glomeruli. Augmented expression of TGFβ in diabetic glomeruli has been correlated with increase in glomerular content of fibronectin and tenascin (41). High-glucose-medium-induced increase in gene and protein expression of collagens I and IV has been shown to be due to autocrine activation of TGFβ in murine mesangial cells (44). Additionally, TGFβ has been shown to increase the synthesis of a variety of CS/DSPG in glomerular epithelial, mesangial, and endothelial cells in vitro (17, 45, 46). Although these data suggest a role for TGFO in glomerular matrix expansion in diabetes, whether reduction related to TGF/β remains to be investigated. Another potential mechanism could involve regulation of glomerular basement membrane HSPG gene expression by high-glucose medium. Preliminary observations in our laboratory suggest that incubation of GEC with 30 mM glucose reduces the mRNA abundance of basement membrane HSPG core protein (47). Further investigation is needed to elucidate the precise mechanism(s) involved in hyperglycemia-induced reduction in synthesis of glomerular basement membrane HSPG in diabetes.

ACKNOWLEDGMENTS

These studies were made possible by grants from the NIH (DK 41517) and Veterans' Administration. The secretarial assistance of Mrs. C. Bryan is gratefully acknowledged. We thank Dr. Y. S. Kanwar for generous donation of the antiHSPG antibody.

REFERENCES

38. Rohrbach, D. H., Wagner, C. W., Star, V. L., Martin, G. R.,