Heat shock protein expression in diabetic nephropathy

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Submitted 2 April 2008; accepted in final form 10 October 2008

Barutta F, Pinach S, Giunti S, Vittone F, Forbes JM, Chiarle R, Arnstein M, Perin PC, Camussi G, Cooper ME, Gruden G. Heat shock protein expression in diabetic nephropathy. Am J Physiol Renal Physiol 295:F1817–F1824, 2008. First published October 15, 2008; doi:10.1152/ajprenal.90234.2008.—Heat shock protein (HSP) HSP27, HSP60, HSP70, and HSP90 are induced by cellular stresses and play a key role in cytoprotection. Both hyperglycemia and glomerular hypertension are crucial determinants in the pathogenesis of diabetic nephropathy and impose cellular stresses on renal target cells. We studied both the expression and the phosphorylation state of HSP27, HSP60, HSP70, and HSP90 in vitro in mesangial cells and podocytes exposed to either high glucose or mechanical stretch. Diabetic and control animals were studied 4, 12, and 24 wk after the onset of diabetes. Immunohistochemical analysis revealed an overexpression of HSP25, HSP60, and HSP72 in the diabetic outer medulla, whereas no differences were seen in the glomeruli. Similarly, exposure neither to high glucose nor to stretch altered HSP expression in mesangial cells and podocytes. By contrast, the phosphorylated form of HSP27 was enhanced in the glomerular podocytes of diabetic animals, and in vitro exposure of podocytes to stretch induced HSP27 phosphorylation via a P38-dependent mechanism. In conclusion, diabetes and diabetes-related insults differentially modulate HSP27, HSP60, and HSP70 expression/phosphorylation in the glomeruli and in the medulla, and this may affect the ability of renal cells to mount an effective cytoprotective response.

mesangial cells; glomerular epithelial cells; mechanical stretch

DIABETIC NEPHROPATHY (DN), the leading cause of end-stage renal failure in the Western world, is characterized by increased albumin excretion rate (AER) and progressive decline in renal function (9, 20). Both hyperglycemia and glomerular capillary hypertension are considered major determinants in the onset and the progression of the complication (5), and in vitro studies on renal cells exposed to high glucose and/or mechanical stretch have partially clarified the underlying cellular mechanisms for this disorder (13).

Heat shock proteins (HSP) are ubiquitous, highly evolutionarily conserved intracellular proteins categorized according to their molecular weight (10). Thermal, oxidative, hemodynamic, osmotic, and hypoxic stresses (17) induce HSP60, HSP27 (human)/HSP25 (rodents), and HSP70 (human)/HSP72 (rodents), and HSP90 expression, and this stress response results in cytoprotection (18). Specifically, HSP prevent non-specific protein assembly, assist in denatured protein refolding, and interfere with proapoptotic pathways. Both hyperglycemia and glomerular capillary hypertension impose cellular stresses on renal target cells and they are thus potential inducers of a stress response that may counterbalance the deleterious effects of these insults. In addition, enhanced expression/phosphorylation of HSP27, an actin-specific molecular chaperone, has been reported in podocytes in experimental nephrotic syndrome (32), suggesting a role for HSP27 in the pathophysiological changes of the podocyte cytoskeleton during the development of proteinuria. Liu et al. (19) have demonstrated that HSP47, a procollagen-specific molecular chaperon, is overexpressed within the glomeruli in both early and advanced diabetic nephropathy, but little is known about the effect of diabetes and diabetes-related insults on other members of this family of proteins.

The present study was designed to assess HSP25/27, HSP60, HSP70/72, and HSP90 expression/phosphorylation states both in vivo in experimental diabetes and in vitro in human podocytes and mesangial cells exposed to either high glucose or mechanical stretch.

MATERIALS AND METHODS

Materials

All materials were purchased from Sigma (St. Louis, MO) unless otherwise stated.

Animal Study

Eight-week-old male Sprague-Dawley rats were used in the present study. All animal procedures were in accordance with the National Health and Medical Research Council of Australia guidelines for animal experimentation. Diabetes was induced by tail-vein injection of streptozotocin (STZ; Boehringer-Mannheim, Mannheim, Germany) at a dose of 50 mg/kg in citrate buffer, following an overnight fast. Control rats were injected with citrate buffer alone. All animals had unrestricted access to water and standard rat chow (Clark-King, Melbourne, Australia). Plasma glucose levels were measured 4 wk after the induction of diabetes, and animals with glucose levels >15 mmol/l 7 days after injection of STZ (>90%) were included in the study. Systolic blood pressure was assessed by tail-cuff plethysmography in prewarmed unanesthetized animals. For determination of urine volume, animals were placed in metabolic cages (Iffa Credo, L’Arbresle, France) for 24 h. Blood samples for measurement of plasma glucose and glycated hemoglobin were collected from the tail. Plasma glucose was measured on an autoanalyzer (ASTRA, Beckman Instruments, Melbourne, Australia). Plasma glucose levels were measured 4 wk after the induction of diabetes, and animals with glucose levels >15 mmol/l 7 days after injection of STZ (>90%) were included in the study. Systolic blood pressure was assessed by tail-cuff plethysmography in prewarmed unanesthetized animals. For determination of urine volume, animals were placed in metabolic cages (Iffa Credo, L’Arbresle, France) for 24 h. Blood samples for measurement of plasma glucose and glycated hemoglobin were collected from the tail vein of conscious rats before the animals were killed. Plasma glucose was measured on an autoanalyzer (ASTRA, Beckman Instruments, Málaga, Spain) with an accuracy of ±0.05 mmol/l.
Fullerton, CA) by the glucose oxidase technique. Glycated hemoglobin was measured by a high-performance liquid chromatography method (Bio-Rad, Richmond, CA) and urinary albumin excretion by radioimmunoassay. Diabetic (n = 18) and control animals (n = 18) were killed 4, 12, and 24 wk following induction of diabetes (6 animals/group at each time point). For tissue collection, animals were anesthetized by intravenous injection of pentobarbital sodium at a dose of 60 mg/kg body wt (Boehringer Ingelheim, Artarmon, New South Wales, Australia). A midline incision of the abdomen was made, and the kidneys were removed and weighed. Half of the right kidneys were fixed in 10% formalin and embedded with paraffin.

**Immunohistochemical analysis.** Immunohistochemical staining was performed on 4-μm paraffin sections of formalin-fixed tissue. Briefly, sections were dewaxed, rehydrated, and immersed in 0.01 M citrate buffer at 100°C for 30 min for retrieval of antigen masked by formalin fixation. Endogenous peroxidase activity was quenched by incubation with 3% H2O2. Endogenous avidin-binding activity was inhibited by sequential treatment with avidin-biotin, and nonspecific binding sites were blocked with 3% BSA. For immunodetection, sections were incubated for 1 h at room temperature with HSP60, HSP72, HSP25, and HSP90 (StressGen, Victoria, BC, Canada), and phospho-HSP25 (Ser15; Acris, Hidenhausen, Germany) antibodies; then, the specific staining was detected using either the LSAB+ system-horseradish peroxidase (HRP) or a rabbit anti-rat biotinylated antibody and streptavidin-HRP (Dako, Glostrup, Denmark). Sections were counterstained and visualized with an epifluorescence microscope (Olympus-Bx4I) connected by a photographic attachment (Leica-DMIL). For each antibody, negative controls were included whereby the primary antibody was replaced with a nonimmune isotypic control antibody. The percent area of staining/number of positive cells was quantified by a computer-aided image-analysis system (Qwin, Leica), whereby 11 randomly selected renal tubulointerstitial fields and 20 glomeruli for each section were analyzed. Evaluations were performed by two independent investigators in a blinded fashion.

**Immunofluorescence microscopy.** Double immunofluorescence was performed on sections from diabetic rats to identify the glomerular cell type overexpressing phospho-HSP25. Synaptopodin and platelet/endothelial cell adhesion molecule-1 (PECAM) were used as markers of podocytes and endothelial cells, respectively. After blocking with 3% BSA, sections were incubated with either an anti-synaptopodin (Progen Biotechnik, Heidelberg, Germany) or an anti-PECAM (Santa Cruz Biotechnology, Santa Cruz, CA) antibody for 18 h at 4°C, washed in PBS, then incubated with FITC-conjugated rat anti-mouse (BD Biosciences, San Jose, CA) or Alexa 555-conjugated donkey anti-goat (Invitrogen, Milan, Italy) antibodies. After washing and further blocking in avidin-biotin, sections were incubated with a rabbit anti-phospho-HSP25 antibody for 1 h at room temperature, followed by 1 h incubation with a biotinylated swine anti-rabbit IgG (Dako, Glostrup, Denmark) and then with either Alexa 555-conjugated streptavidin or Alexa 480-conjugated streptavidin (Invitrogen). Sections were examined using an Olympus epifluorescence microscope (Olympus Bx4 I) with photographic attachment (Leica-DMIL). The images were color-combined and assembled into photomontages by using Adobe Photoshop (Universal Imaging, West Chester, PA).

**In Vitro Study.**

**Cell culture.** Cultures of immortalized human podocytes and mesangial cells were established and characterized as previously described (2, 12). Cells were cultured in DMEM (Invitrogen) containing l-glutamine, 6.8 mM glucose, 10–20% heat-inactivated fetal calf serum (Euroclone Milan, Italy), 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified incubator at 37°C. ***Application of mechanical stretch to cultured cells.*** Podocytes and mesangial cells were seeded in equal number into six-well type I collagen-coated silicon elastomer-base culture plates (Flex I plates) and control plates (Flex II plates, Flexcell International, McKeesport, PA). After serum deprivation for 24 h, cells were subjected to repeated stretch/relaxation cycles by mechanical deformation using a stress unit, which consists of a vacuum unit and a baseplate (14). A vacuum was cyclically applied (60 cycles/min) to the rubber base via the baseplate, which was placed in a humidified incubator with 5% CO2 at 37°C. Cells were exposed to an average 10% uniaxial elongation, which mimics that present in vivo in glomeruli exposed to supernormal pressure levels. Control cells were grown in nondeformable but otherwise identical plates (Flex II plates). We applied a cyclical mechanical stretch on the evidence that, in the normal glomerulus, capillary pressure is pulsatile and that, in situations such as diabetes, this pulsatility may be enhanced because of defective autoregulation.

**Podocyte and mesangial cell exposure to high glucose concentrations.** After serum deprivation for 24 h, human podocytes and mesangial cells were exposed to either high (25 mM) or normal (6.8 mM) glucose concentrations for 1, 3, or 5 days. Media were made isosmolar with the addition of mannitol.

**Glomerular cell exposure to heat stress.** Podocytes and mesangial cells were deprived for 24 h in culture flasks, which were then sealed by wrapping of parafilm around their lids. The flasks were immersed in a thermostatted water bath at 37, 40, 42, and 44 ± 0.5°C for 30 min. Cells were then resuspended in fresh medium and allowed to recover for 6 h at 37°C to permit synthesis and accumulation of HSP.

**Western blotting.** Cells were harvested in a Tris (50 mM, pH 7.4) lysis buffer containing 150 mmol/l NaCl, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 mmol/l NaF, 1% Nonidet P-40, 2 mmol/l EGTA, and 1 mmol/l PMSF. Thirty micrograms of total protein extracts were separated by either 10% (HSP60, HSP70) or 12% (HSP27, P38) PAGE and electrotransferred to nitrocellulose membranes. The membranes were blocked in 5% skim milk in Tris-buffer-Tween 20 (pH 7.6) and subsequently incubated with mouse anti-HSP60, mouse anti-HSP70, rabbit anti-HSP27, rabbit anti-phospho-HSP27 (Ser82; StressGen), rabbit anti-P38 (Promega, Southampton, UK), or rabbit anti-phospho-P38 (Insight Biotechnology, Wembley, UK) antibodies for 1 h at room temperature. After washing in Tris-buffer-Tween 20, membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature (GE Healthcare, Buckinghamshire, UK). Detection was performed by enhanced chemiluminescence reagents (Amersham), and the band intensity was quantified by laser densitometry. Equal protein loading of each lane was verified with Ponceau staining of total proteins on the nitrocellulose membranes.

**Data Presentation and Statistical Analysis.**

The number of experiments, carried out in at least triplicate, is reported. All data are presented as means ± SE and expressed as fold-change over control. Student’s t-test was used for the comparison between two groups. Values of P < 0.05 were considered significant.

**RESULTS.**

**Metabolic and Physiological Parameters.**

Diabetes was associated with reduced body weight and elevations in both plasma glucose and glycated hemoglobin levels. Albumin excretion rate and blood pressure were also significantly higher in diabetic rats compared with control animals (Table 1).

**Effect of Early Diabetes on HSP Expression in Renal Medulla.**

To assess whether diabetes induces changes in renal HSP expression, we compared immunohistochemical staining for HSP60, HSP72, HSP25, and HSP90 in kidney sections from diabetic and control (nondiabetic) rats 4, 12, and 24 wk after diabetes induction. Antibody specificity was confirmed by disap-
appearance of the signals when isotype-matched control antibodies were used as primary antibodies. At all time points, expression of HSP25, HSP60, and HSP72 was significantly enhanced, exclusively in the outer medulla, in diabetic rats compared with control animals (Fig. 1, A–L). HSP72 staining localized predominantly at the tubular level, while HSP25 immunoreactivity was found primarily in the interstitial vessels. HSP90 was highly expressed in renal medulla, but staining intensity was similar in diabetic and nondiabetic animals (Fig. 1, M–P).

**Effect of Diabetes and Diabetes-Related Insults on HSP Expression in Glomerular Cells**

There was no significant difference in glomerular HSP25, HSP60, and HSP90 expression between control and diabetic animals. No glomerular cell stained positively for HSP60 in either control or diabetic animals (Fig. 2, A–E). By contrast, HSP25 and HSP90 were strongly expressed within the glomeruli, with a staining distribution suggestive of localization to both mesangial cells and podocytes, but their expression was not altered by the presence of diabetes (Fig. 2, K–O, P–T). Staining for HSP72 was greater in 4-wk diabetic rats than in control animals (diabetic 1.66 ± 0.66 vs. nondiabetic 0.2 ± 0.06, P < 0.05) (Fig. 2, F and G). However, the absolute number of HSP72-positive glomerular cells was modest (1–2/glomerulus), and no differences were observed at later time points (Fig. 2, H and I).

Similarly, in vitro exposure of both podocytes and mesangial cells to diabetes-related insults, either to high glucose or mechanical stretch, did not alter the expression of these HSP. As shown

| Table 1. General assessment parameters in each of the study groups |
|----------------|----------------|----------------|----------------|----------------|
|               | Body Weight, g | Glucose, mmol/l | Glycated Hb, % | Blood Pressure, mmHg | Albumin Excretion, µg/day |
| Nondiabetic (4 wk) | 446±8          | 6.2±0.2         | 3.7±0.1        | 128±3          | 0.4 ×/× 1.2 |
| STZ-diabetic (4 wk) | 331±9*         | 28.8±0.7*       | 13.8±0.3*      | 145±7§         | 1.11 ×/× 1.1* |
| Nondiabetic (12 wk) | 453±8          | 5.4±0.2         | 3.6±0.2        | 133±3          | 3.1 ×/× 1.17 |
| STZ-diabetic (12 wk) | 302±15‡       | 22.1±1.7‡       | 10.6±0.4‡      | 147±3§        | 15.8 ×/× 1.24§ |
| Nondiabetic (24 wk) | 743±19         | 6.9±0.3         | 3.1±0.1        | 127±3         | 3.2 ×/× 0.8 |
| STZ-diabetic (24 wk) | 312±21†        | 27.3±2.5†       | 14.3±0.8†      | 147±4†        | 18.7 ×/× 10.0† |

Values are means ± SE, except for albumin excretion, which is shown as geometric mean ×/× tolerance factor. *P < 0.001, †P < 0.01, ‡P < 0.005, §P < 0.05: vs. age-matched nondiabetic mice.
in Fig. 3, HSP27, HSP60, and HSP70 were highly expressed by both human mesangial cells and podocytes in the basal condition. However, expression of HSP27, HSP60, and HSP70 was not changed by exposure to either stretch for 6, 12, and 24 h or high glucose for 1, 3, and 5 days. By contrast, exposure of podocytes to thermal stress significantly enhanced both HSP27 and HSP70 expression, with a peak threefold increase at 44°C. As shown in Fig. 4, a similar trend was also observed in mesangial cells.

Glomerular Phospho-HSP25 Expression

Phospho-HSP25 was detectable in the glomeruli of control rats, and staining intensity was substantially enhanced in the diabetic animals (Fig. 5, A–F). Semiquantitative analysis showed a peak, greater than fourfold increase at 12 wk (nondiabetic 3.05 ± 0.8 vs. diabetic 12.9 ± 1.7, percentage area, P < 0.05), but a significant increase was also observed at both 4 (nondiabetic 3.9 ± 0.3 vs. diabetic 10.3 ± 0.5, P < 0.001) and 24 wk (nondiabetic 6.84 ± 1.47 vs. diabetic 19.12 ± 2.1, P < 0.05). In the diabetic rats, phospho-HSP25 staining showed a comma-like pattern along the glomerular capillary wall suggestive of podocyte localization. 

Based on this presumed localization, double-labeling immunofluorescence was performed and colocalization of phospho-HSP25 with synaptopodin, a specific podocyte marker, was observed around the lumen of the glomerular capillaries, resulting in a partial yellow overlap. This indicates that phospho-HSP25 was primarily expressed by glomerular podocytes, although phospho-HSP25 and synaptopodin differ with respect to their intracellular distribution. No overlap was seen with PECAM, a marker of glomerular endothelial cells (Fig. 5, G–L).

Stretch Induces HSP27 Phosphorylation in Glomerular Epithelial Cells

Having established that diabetes enhances phospho-HSP25 expression in glomerular podocytes in vivo, we investigated in vitro the underlying potential cellular mechanisms. Previous studies have shown that podocyte exposure to high glucose induces HSP27 phosphorylation (6); but the role of mechanical stretch, mimicking glomerular capillary hypertension, is still unknown; therefore, we investigated the effect of stretch on the HSP27 phosphorylation state. Serum-deprived podocytes were
exposed to stretch for 5, 10, 30, and 60 min, and then expression of both phospho-HSP27 and total HSP27 was assessed by immunoblotting. Phosphorylated HSP27 was almost undetectable in the basal condition, but exposure to stretch induced a rise in phospho-HSP27 levels by 5 min, with a maximum 4.4-fold increase at 10 min. High levels were sustained for up to 1 h. Total HSP27 protein levels remained unchanged in stretched cells (Fig. 6, A and B).

P38 MAPK Mediates Stretch-Induced HSP27 Phosphorylation

The P38-MAPKAP2 signaling pathway plays a key role in HSP27 phosphorylation in various cell types (7). To test whether stretching of podocytes induces P38 phosphorylation, we exposed podocytes to stretch for short time periods (5, 10, 30, and 60 min) and assessed phospho-P38 levels by immunoblotting. We found that stretch did not alter total P38 protein levels but induced a significant twofold increase in activated P38 levels after 5 min with a return to the basal level by 60 min (Fig. 6C). To assess whether P38 was the mediator of stretch-induced HSP27 phosphorylation, podocytes were exposed to stretch for 10 min either in the presence or in the absence of SB202190 (1 μM), a compound that specifically inhibits P38 by binding to its ATP pocket (35). We found that the addition of SB202190 completely abolished stretch-induced HSP27 phosphorylation at 10 min (Fig. 6D), providing evidence that the increase in phospho-HSP27 in response to stretch occurred via a P38-dependent mechanism.

DISCUSSION

The majority of the studies on diabetic complications have been performed to identify insults implicated in cell injury and to clarify intracellular mechanisms of cell damage, but little has been done to assess the potential role of dysfunction of cellular machineries involved in cytoprotection. In this study, we investigated both in vivo in experimental diabetes and in vitro in glomerular cells exposed to diabetes-related insults the expression of members of the HSP family playing a pivotal role in cell protection.

In control animals, intrarenal expression of HSP25, HSP60, HSP70, and HSP90 was similar to that previously described by others in the rat kidney (25). HSP60 was predominantly expressed in the cortex and in the outer medulla, with a distribution that mirrors the abundance of mitochondria as HSP60 localizes primarily in the matrix space of mitochondria (1). Expression of both HSP25 and HSP72 increased progressively...
along the corticopapillary axis and paralleled the osmotic gradient. Indeed, HSP25 and HSP72 are induced by hyperosmolarity and they assist in the adaptation of medullary cells to high extracellular solute concentrations (1). HSP90 was mainly expressed in the collecting ducts, where it is known to interact with steroid hormone receptors.

In both early and advanced experimental diabetes, there was a significant increase in HSP25, HSP60, and HSP72 expression in the outer medulla, whereas no changes were seen in HSP90 expression. Consistent with our results, a recent report has showed HSP60 upregulation in total protein extracts from diabetic rat kidneys (3, 31). The distribution of HSP72 staining was suggestive of localization to both collecting ducts and thick ascending limbs, while HSP25 was overexpressed by the interstitial vessels, possibly in the pericytes surrounding the descending vasa recta, which are known to have a highly structured actin cytoskeleton (22). Although STZ is known to induce tubular damage, this occurs predominantly in cells of the S1 portion of the proximal tubuli, which are located in the renal cortex (29, 37). It is, thus, unlikely that the increase in HSP expression we observed in the diabetic outer medulla was secondary to STZ-induced tubular injury. In both human and experimental diabetes, there is a reduction in oxygen tension, particularly in the outer medulla, and overexpression of tubular carrier proteins in outer medullary thick ascending limbs, which enhance sodium chloride reabsorption and interstitial osmolarity (11, 33). The increased hypertonic and hypoxic stress in the diabetic outer medulla may induce an overexpression of HSP localized specifically to this area, which, in turn, may result in cytoprotection and counterbalance diabetes-induced cell injury. Several studies have reported that nephrotoxicity due to nephrotoxic agents, such as gentamicin, and cisplatin, is reduced in diabetic animals (30, 34), but the underlying mechanism remains unknown. Our results raise the

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**Fig. 5.** Phosphorylated form of HSP25 (P-HSP25) is increased in the glomeruli from diabetic rats. Glomerular immunohistochemistry for phospho-HSP25 in DM (D–F) and ND (A–C) rats at 4 (A and D), 12 (B and E), and 24 wk (C and F). Immunofluorescence for phospho-HSP25 (G and J), synaptopodin (K) and platelet/endothelial cell adhesion molecule-1 (PECAM; H) in diabetic glomeruli. Phospho-HSP25 colocalized with synaptopodin (L), while no overlap (I) was observed with PECAM. Magnification ×400.
vascular smooth muscle cells (36). Furthermore, our observation in other cell types such as tubular epithelial cells (26) and cells as stretch has been shown to induce HSP70 overexpression (32) and linked to failure in cardiovascular protection (15, 27). Our organs of diabetic complications has been previously reported in diabetes, and associated with a lack of HSP expression. Accordingly, previous studies have shown that diabetes-related insults. In control rats, both HSP25 and HSP90 were strongly expressed within the glomeruli, predominantly by podocytes; whereas no staining for HSP60 and HSP72 was found. Podocytes have a highly structured actin cytoskeleton (24) that plays a key role in the control of glomerular permesselectivity, and this may provide an explanation for the high expression of the actin binding protein HSP25 in this cell type. Comparison of glomerular HSP staining in control and diabetic rats 4, 12, and 24 wk after the induction of diabetes showed that HSP25 and HSP90 expression does not differ in diabetic and nondiabetic animals and that HSP60 is undetectable in both. HSP72-positive glomerular cells were found in diabetic rats 4 wk after the induction of diabetes, but their absolute number was very modest and this effect was transient as it was no longer present at 12 and 24 wk. No such HSP72-positive cells were observed in glomeruli from control rats. A lack of HSP60 and HSP70 induction in response to stress in target organs of diabetic complications has been previously reported and linked to failure in cardiovascular protection (15, 27). Our results extend this finding to the diabetic glomeruli and raise the possibility that the inability of the diabetic glomeruli to mount an effective stress response may contribute to explain the possibility that the inability of the diabetic glomeruli to mount an effective stress response is limited to diabetes-related insults. By contrast, we found a significant increase in the phosphorylated form of HSP25 in the glomeruli from diabetic animals compared with controls. This increase was observed in both early and more advanced diabetes and peaked 12 wk after induction of diabetes. Both the pattern of staining and co-staining with the podocyte marker synaptopodin suggest a localization to the podocytes. Previous studies have reported an increase in the phosphorylated form of HSP25 in the diabetic glomeruli (6, 21, 23), but this is the first study that identifies the specific glomerular cell type involved. Furthermore, our in vitro data show that podocyte exposure to stretch, mimicking glomerular capillary hypertension, induces a rapid and significant 4.4-fold increase in the levels of phosphorylated HSP27. This, together with previous reports showing HSP27 phosphorylation in podocytes exposed to high glucose (6), suggests a potential cellular mechanism for the increase in the levels of phospho-HSP27 we observed in experimental diabetes. Stretch-induced HSP27 phosphorylation was mediated by a P38-dependent mechanism as the application of stretch induced a significant twofold increase in activated P38 levels, with P38 inhibition completely abolishing HSP27 phosphorylation. Similarly, high glucose-induced HSP27 phosphorylation is P38 dependent in podocytes (6), and a P38-MAPK-activated protein kinase 2/3 (MK2/3) pathway has been implicated in HSP27 phosphorylation in various cell types (4).

HSP27 phosphorylation is required for polymerization of the actin cytoskeleton, which is highly expressed in the podocyte foot processes. The observation that in experimental nephrotic syndrome foot process effacement and proteinuria are paralleled by enhanced HSP27 phosphorylation in the foot processes (32) and that in vitro TNF-α enhances transendothelial permeability via a P38-phospho-HSP27 mechanism (16) has led to the hypothesis that HSP27 phosphorylation plays a role.
in the pathogenesis of the diabetic proteinuria by affecting foot process architecture. In contrast, Park et al. (23) have recently reported that albuminuria is not ameliorated in diabetic mice knockout for MK2. However, in this study HSP27 phosphorylation was only partially prevented, and it is known that not only MK2, but also MK3 can phosphorylate HSP27 on the same serine residues (4). Strategies that specifically block HSP27 phosphorylation are required to clarify the potential pathophysiological relevance of HSP induction in the diabetic glomeruli may contribute to counterbalance diabetes-induced cell damage. By contrast, lack of HSP induction in the diabetic glomeruli may contribute to the susceptibility of glomerular cells to injury. Further studies are required to clarify the potential pathophysiological relevance of HSP27 phosphorylation in the pathogenesis of diabetic renal disease.

GRANTS

This work was supported by the “Compagnia di San Paolo”, the Piedmont Region, and the University of Turin as well as by a Centre Grant from the Juvenile Diabetes Research Foundation (to M. E. Cooper).

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