Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress in rat kidney cortex

Yizhen Xu, Brent W. Osborne, and Robert C. Stanton

Renal Division, Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts

Submitted 23 February 2005; accepted in final form 6 June 2005

Xu, Yizhen, Brent W. Osborne, and Robert C. Stanton. Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress in rat kidney cortex. Am J Physiol Renal Physiol 289: F1040–F1047, 2005. First published June 14, 2005; doi:10.1152/ajprenal.00076.2005.—The incidence of diabetic nephropathy has been increasing. Studies have shown that oxidative stress (due to increased oxidant production and/or decreased antioxidant activity) is a critical underlying mechanism. The principal intracellular reductant is NADPH whose production is mainly dependent on glucose-6-phosphate dehydrogenase (G6PD) activity. Our work in cultured cells previously showed that high glucose caused activation of protein kinase A (PKA) and subsequent phosphorylation and inhibition of G6PD activity and hence decreased NADPH (Zhang Z, Apse K, Pang J, and Stanton RC. J Biol Chem 275: 40042–40047, 2000). The purpose of this study was to determine whether these findings occur in diabetic rats (induced by streptozotocin) compared with control. G6PD activity and accordingly NADPH levels and glutathione levels were significantly decreased in diabetic kidneys compared with control kidneys. Lipid peroxidation was significantly increased, which correlated with decreased G6PD activity ($r = 0.48$). G6PD expression was significantly reduced, which correlated with decreased G6PD activity ($r = 0.72$). PKA activity and serine phosphorylation of G6PD were significantly increased and were closely correlated with decreased G6PD activity ($r = 0.51$ for PKA activity; $r = 0.93$ for serine phosphorylation of G6PD). Insulin treatment and/or correction of hyperglycemia ameliorated the changes caused by diabetes. In conclusion, chronic hyperglycemia caused inhibition of G6PD activity via decreased expression and increased phosphorylation of G6PD, which therefore led to increased oxidative stress.

diabetic nephropathy; pentose phosphate pathway

Diabetes is the leading cause of renal failure in the United States, accounting for 40% of the new cases each year (37, 45). The mechanisms responsible for the pathogenesis of diabetic nephropathy are not fully understood. Advanced glycation end products (AGEs), activation of protein kinase Cβ (PKC), activation of aldose reductase, and others have been shown to play important roles (4, 40). Recently, much attention has been focused on increased oxidative stress (17, 31). Both increased production of oxidants and decreased actions of antioxidants have been shown to play roles in the increased oxidative stress of diabetic nephropathy.

Glucose-6-phosphate dehydrogenase (G6PD) is the first and rate-limiting enzyme of the pentose phosphate pathway, which results in the production of ribose-5-phosphate and NADPH (Fig. 1). The entire antioxidant system, as well as other reductant-requiring processes, relies on an adequate supply of NADPH because it is the principal intracellular reductant for all cells. We and others (11, 20, 21, 29, 30) have shown that G6PD is the principle source of NADPH. Therefore, a decrease in G6PD activity leads to decreased NADPH and makes cells very sensitive to oxidant damage. For example, Martini and Ursini (32) indicated that partial or complete inhibition of G6PD activity led to highly significant increases in oxidative stress and cell death compared with results shown in wild-type cells. Also Rosenstrauss and Chasin (38), using a G6PD-deficient Chinese hamster ovary cell line, showed that G6PD-deficient cells were more susceptible to oxidative stress. Moreover, Pandolfi et al. (36), using homologous recombination in mouse embryonic stem cells to produce a cell line totally deficient in G6PD, showed that the G6PD-null cells were exquisitely sensitive to oxidative stress, as seen by much higher percentages of cell deaths at low oxidant concentrations compared with that shown in wild-type cells. In addition, the G6PD-null cells had significantly reduced cloning efficiencies and decreased growth rates compared with that shown in wild-type cells expressing G6PD (36). These results have provided strong evidences showing that NADPH, which is mainly produced by G6PD, is of central importance to cellular redox regulation. On the other hand, any changes in G6PD activity will alter NADPH levels and thus impact the antioxidant system. Recently, our group and others (1, 49) reported that G6PD activity was inhibited in endothelial cells exposed to high glucose and that this inhibition of G6PD occurred in part via phosphorylation caused by high glucose-induced protein kinase A (PKA) activation (49).

From our previous work, we hypothesize that diabetes causes inhibition of G6PD activity in experimental animal models and that this inhibition is mediated at least in part through activation of PKA. As a result, decreased G6PD activity will lead to lower intracellular NADPH levels and therefore increased oxidative stress. In this study, we examined G6PD activity and NADPH levels, as well as oxidative stress markers [lipid peroxidation, glutathione (GSH) levels, and activities of glutathione reductase, glutathione peroxidase (GPX), catalase, and superoxide dismutase (SOD)] in the kidney cortices from experimental diabetic animals. To evaluate the mechanisms responsible for high glucose-induced G6PD inhibition, PKA activity, phosphorylation of G6PD, and expression of G6PD were also determined.

MATERIALS AND METHODS

Animal experimental protocols. Male Sprague-Dawley rats (Taconic Farms) weighing 240–260 g were maintained on a standard diet and housed in a temperature-controlled environment with controlled lighting and ad libitum access to food and water. Animals were assigned randomly to one of two groups and then fasted overnight. The following day, animals were injected at 24 h intervals with streptozotocin (STZ; 55 mg/kg body weight) or an equal volume of vehicle (0.1 M acetic acid with 2% ascorbic acid). Groups included: non-diabetic control (vehicle, five animals), non-diabetic diabetic (vehicle, five animals), streptozotocin control (five animals), streptozotocin diabetic (five animals). After 7 days, animals were killed by exsanguination under anesthesia, and kidneys were removed, weighed, and snap frozen in liquid nitrogen. Kidney cortex was dissected under sterile conditions on ice, and samples were homogenized in ice cold homogenization buffer (100 mM Hepes, 250 mM sucrose, 5 mM MgCl2, 10 mM KCl, 1 mM EDTA, 1 mM DTT, pH 7.4) using a polytron with a micro tissue homogenizer. Homogenates were centrifuged at 11,000 g for 15 min at 4°C to pellet debris. Following centrifugation, supernatants were collected and stored at −80°C. Glucose-6-phosphate dehydrogenase activity was determined by measuring the production of ribose-5-phosphate and NADPH from glucose-6-phosphate according to previously described methods (36). Glutathione (GSH) levels were determined by the DTNB assay adapted from Ellman (9). Glutathione peroxidase activity was determined by an O2 uptake coupled assay (26). Glutathione reductase activity was determined by the NADPH oxidation coupled assay method (37). Glutathione S-transferase activity was determined by measuring the formation of NADPH, as described recently by Hwang et al. (19).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Institutional Animal Care and Use Committee of the Joslin Diabetes Center. All animal methods and protocols were approved by the Institutional Animal Care and Use Committee of the Joslin Diabetes Center. Diabetes mellitus was induced by an intraperitoneal injection of streptozotocin (Sigma, St. Louis, MO) in citrate buffer (0.1 mol/l, pH 4.5) with a dose of 55 mg/kg body wt followed by an overnight fast. The non-diabetic (NDM) control group received injection of a citrate buffer alone. Blood glucose levels were determined 2 days after streptozotocin injection, and the rats with blood glucose levels higher than 250 mg/dl were considered diabetic. The diabetic rats were then randomly divided into two subgroups: diabetic rats and diabetic rats treated with insulin. For the insulin-treated diabetic rat groups, insulin pellets (Linshin Canada, Ontario, Canada) were implanted subcutaneously after 48 h, following the onset of diabetes. The pellet releases regular insulin at a constant rate of 2 U/24 h for 40 days, after which a new pellet must be implanted to achieve glucose control. Body weights and blood glucose concentrations were determined weekly in all animals. Rats from the three different groups were euthanized at the 1st, 4th, 8th, and 16th wk postinjection, respectively. For each time period, the NDM, diabetic, and diabetic rats treated with insulin were weighed and then sliced very thinly and thawed in RIPA buffer, which consisted of PBS, 1% Nonidet P-40, 0.1% sodium deoxycholate, 0.1% SDS, 10 µg/ml PMSF, 50 KIU/ml aprotinin, and 10 µl/ml sodium orthovanadate. Tissues were further disrupted and homogenized by a Dounce homogenizer and then were centrifuged at 10,000 g for 10 min at 4°C. Supernatant was removed, and protein concentration of cell lysates was measured by using the Bio-Rad protein assay kit. Anti-rabbit IgG beads (50 µl; eBioscience) were added again, and the resultant mixture was incubated at 4°C for 1 h. The IgG bead-antibody complex was isolated by centrifugation and washed with 500 µl of lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40) for three times. After the last wash, supernatant was aspirated completely, and the IgG bead-antibody complex was then treated with 100 µl of SDS gel loading buffer and boiled for 5 min to dissociate the IgG-antibody complexes. Western blot analysis.

Western blot analysis. Western blot assay was conducted as previously described (15). Precleared tissue lysates as well as immunoprecipitation products were resolved by 10% SDS-PAGE and then transferred to a nitrocellulose membrane. Membranes were incubated in SuperBlock blocking buffer (Pierce, Rockford, IL) for 1 h at room temperature. Subsequently, the membranes were hybridized with rabbit anti-G6PD antibodies (1:1,000 dilution; Bethyl Laboratories), mouse monoclonal anti-phosphoserine antibodies (1:100 dilution; Calbiochem), or β-actin antibody (1:1,000 dilution; Santa Cruz, Santa Cruz, CA) overnight at 4°C. After being washed three times for 10 min each with Tris-HCl (pH 8.0) containing 0.1% Tween 20, the membranes were incubated in HRP-conjugated goat anti-rabbit antibodies or goat anti-mouse antibody solution (1:1,000 dilution; Bio-Rad) at room temperature for 1 h. After antibody incubation, immunodetection was performed with the enhanced chemiluminescence-Western blotting detection system (Amersham Biosciences, Piscataway, NJ) according to the manufacturer’s protocol.
Results of the study are presented in Table 1. Animal characteristics. Blood glucose, body weight, and kidney weight were measured at 1, 4, 8, and 16 weeks in nondiabetic (NDM) and diabetic animals. Insulin treatment normalized the changes in blood glucose levels, lower body weight, and elevated kidney/body weight in diabetic animals. Implantation of insulin pellets allowed normalization of blood glucose levels in the insulin-treated diabetic group compared with their counterparts in the diabetic group. In all groups, however, there was a highly significant increase in blood glucose levels compared with nondiabetic animals (Table 1).

G6PD activity and NADPH level. G6PD activity was decreased in diabetic animals compared with NDM controls (Fig. 2A). The change was of statistical significance from groups at 4, 8, and 16 weeks. Insulin treatment normalized the decrease in G6PD activity in diabetic animals. The NADPH level in diabetic animals was also decreased compared with NDM animals (Fig. 2B). The difference was of statistical significance from groups at 4, 8, and 16 weeks. Of note, the changes of G6PD activity for NADPH levels in diabetic animals are similar to those seen in cultured bovine aortic endothelial cells exposed to high glucose, as previously reported by our group (49). Insulin treatment normalized the significant decrease of the NADPH level in diabetic animals (Fig. 2B), suggesting that improvement in the blood glucose levels and/or administration of insulin alone might lead to an normalization in G6PD activity.

Oxidative stress markers. TBARS levels were measured as an index of malondialdehyde production and hence lipid peroxidation. Results indicated a significant increase of TBARS in diabetic animals at 8 and 16 weeks of diabetes duration compared with NDM control animals (Fig. 3A). Insulin treatment normalized (week 8) or ameliorated (week 16) the increase of TBARS level. These results show, as demonstrated by other investigators, that diabetes leads to increased oxidative stress. Further evaluations of enzymes and chemicals associated with antioxidant system were done. As shown in Fig. 1, the normal function of GSH coupling relies on a sufficient supply of NADPH. Thus a decrease in NADPH should lead to decreased GSH levels. Figure 2C shows that GSH levels were significantly decreased in animals with long (8 and 16 weeks) diabetes duration compared with NDM control animals. Insulin treatment normalized the significant decrease of GSH level in diabetic animals (Fig. 2C). Examination of other enzymes associated with oxidant stress (glutathione reductase, catalase, and SOD) did not show any significant differences between diabetic and NDM control animals (Table 2).

PKA activity and phosphorylation of G6PD. Previous work from our laboratory (49) has shown that high glucose led to increased PKA activity and phosphorylation of G6PD in cultured bovine aortic endothelial cells. Further studies with the catalytic subunit of PKA in vitro showed that PKA can directly phosphorylate G6PD and inhibit G6PD activity (49). Thus studies were done to determine whether kidneys from diabetic animals also showed increased PKA activity. Figure 4A shows that PKA activity from diabetic groups was significantly increased compared with nondiabetic animals at 16 weeks after diabetes induction. These results show that there is increased oxidative stress in the kidney cortex, as reported by other laboratories (17, 31).

Table 1. Animal characteristics

<table>
<thead>
<tr>
<th></th>
<th>1 week</th>
<th>4 week</th>
<th>8 week</th>
<th>16 week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood glucose, mg/dl</td>
<td>Body weight, g</td>
<td>Ratio of kidney weight to body weight, mg/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>83.40±2.13</td>
<td>270.01±4.50</td>
<td>9.46±0.08</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>386.40±25.76*†</td>
<td>223.00±8.39*†</td>
<td>15.86±0.16*†</td>
<td></td>
</tr>
<tr>
<td>Diabetic with insulin treatment</td>
<td>155.80±8.86*</td>
<td>243.20±5.54*</td>
<td>10.88±0.34*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 rats in each group. *P < 0.05 compared with nondiabetic controls; †P < 0.05 compared with diabetic with insulin treatment.
animals, then there should be evidence of serine/threonine phosphorylation of G6PD from rat kidney cortex. Using antibodies to G6PD to immunoprecipitate and using blotting with anti-phosphoserine antibodies, we show (Fig. 4, B and C) that serine phosphorylation levels of G6PD in diabetic animals were increased compared with NDM control animals. Insulin treatment normalized the change in G6PD phosphorylation in diabetic animals. The data shown in Fig. 4 were obtained from animals from week 16. Data not shown here indicated that the same holds true in experimental animals at 4 and 8 wk. Although increased serine phosphorylation of G6PD does not necessarily have to be due to PKA, Fig. 4D shows that there is a significant correlation between phosphorylation of G6PD activity and the level of PKA activity ($r = 0.51$, $n = 9$, $P < 0.01$), suggesting that increased PKA activity is at least a major factor in the increased phosphorylation of G6PD. Moreover, Fig. 4E shows that there is a very close correlation ($r = 0.93$, $n = 9$, $P < 0.001$) between phosphorylation of G6PD and inhibition of G6PD activity, suggesting that phosphorylation plays a role in the inhibition of G6PD observed in the kidney cortex of diabetic animals.

Expression of G6PD. In addition to posttranslational modification of G6PD, decreases in G6PD expression can also lead to decreased activity. To explore this possibility, lysates of kidney cortex were isolated from diabetic and NDM animals. Each sample was resolved by SDS-PAGE and visualized using antibodies to G6PD by Western blot analysis. All data were normalized to expression of actin. The data in Fig. 5, A and B, show that expression in diabetic animals was significantly reduced compared with NDM animals. Insulin treatment normalized the change in G6PD expression in diabetic animals. The data in Fig. 5 are from the 16-wk groups. Data not shown here suggested the same held true in the 4- and 8-wk groups. Moreover, Fig. 5C shows that there was a significant correlation between G6PD activity and expression of G6PD ($r = 0.72$, $n = 9$, $P < 0.05$).

Fig. 2. A: G6PD activities in kidney cortices of nondiabetic control (NDM), diabetic (DM), and insulin-treated diabetic (INS) rats. B: NADPH levels in kidney cortices of NDM, DM, and INS rats. C: GSH levels in kidney cortices of NDM, DM, and INS rats. Data are means ± SE of 5 rats in each group. *$P < 0.05$ vs. NDM group; †$P < 0.05$ vs. INS group.

Fig. 3. A: thiobarbituric acid reactive substance (TBARS) levels in kidney cortices of NDM, DM, and INS rats. Data are means ± SE of 5 rats in each group. B: correlation of TBARS levels to the G6PD activity, which was calculated from values shown in Figs. 2A and 3A. Correlation coefficient was calculated by linear regression analysis. *$P < 0.05$ vs. NDM group; †$P < 0.05$ vs. INS group.
Together, these results suggest that both phosphorylation of G6PD and decreased protein expression mediate the diabetes-induced decrease of G6PD.

**DISCUSSION**

Many studies have shown that high glucose leads to increased oxidative stress in kidneys from diabetic animals or patients (16, 17, 28). The evidence for increased oxidative stress in diabetic complications is extensive, and some examples are as follows.

1) Cultured cells exposed to high glucose have increased oxidative stress as determined by increased levels of lipid peroxidation and increased levels of reactive oxygen species (14, 18).
2) Cultured cells treated with increased levels of intracellular antioxidants such as GSH, SOD, or catalase are protected against deleterious effects of high glucose (14).
3) Increased reactive oxygen species have been shown either to cause or to be a consequence of other mechanisms that have been shown to mediate diabetic complications. For example, increased aldose reductase activity leads to depletion of NADPH and, as a consequence, increased intracellular reactive oxygen species (14).

PKC activation can occur as a result of increased oxidative stress (8), and PKC activation levels of lipid peroxidation and increased levels of reactive oxygen species (14, 18).

**Table 2. Antioxidant enzyme activity in kidney cortices from nondiabetic, diabetic, and diabetic with insulin treatment animals**

<table>
<thead>
<tr>
<th></th>
<th>1 week</th>
<th>4 week</th>
<th>8 week</th>
<th>16 week</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glutathione peroxidase, nmol min⁻¹ mg protein⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>14.37 ± 0.74</td>
<td>13.02 ± 0.98</td>
<td>14.41 ± 1.05</td>
<td>14.08 ± 0.73</td>
</tr>
<tr>
<td>Diabetic</td>
<td>10.69 ± 1.55*</td>
<td>14.66 ± 2.51</td>
<td>12.89 ± 1.59</td>
<td>17.45 ± 2.83</td>
</tr>
<tr>
<td>Diabetic with insulin treatment</td>
<td>15.44 ± 0.72</td>
<td>14.97 ± 0.22</td>
<td>13.81 ± 0.98</td>
<td>15.01 ± 1.53</td>
</tr>
<tr>
<td><strong>Glutathione reductase, nmol min⁻¹ mg protein⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>1.83 ± 0.04</td>
<td>1.86 ± 0.04</td>
<td>2.09 ± 0.07</td>
<td>1.94 ± 0.07</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.61 ± 0.07</td>
<td>1.68 ± 0.07</td>
<td>1.85 ± 0.03</td>
<td>1.81 ± 0.07</td>
</tr>
<tr>
<td>Diabetic with insulin treatment</td>
<td>1.56 ± 0.13</td>
<td>1.75 ± 0.05</td>
<td>1.88 ± 0.08</td>
<td>1.77 ± 0.07</td>
</tr>
<tr>
<td><strong>Catalase, nmol min⁻¹ mg protein⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>49.02 ± 1.79</td>
<td>41.56 ± 1.88</td>
<td>42.62 ± 3.72</td>
<td>37.32 ± 1.32</td>
</tr>
<tr>
<td>Diabetic</td>
<td>41.32 ± 4.07</td>
<td>41.86 ± 2.87</td>
<td>43.80 ± 4.37</td>
<td>38.56 ± 2.91</td>
</tr>
<tr>
<td>Diabetic with insulin treatment</td>
<td>41.69 ± 3.83</td>
<td>40.10 ± 2.63</td>
<td>42.03 ± 1.16</td>
<td>34.71 ± 1.89</td>
</tr>
<tr>
<td><strong>Superoxide dismutase, U/mg protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>15.52 ± 3.12</td>
<td>14.84 ± 1.22</td>
<td>16.77 ± 1.80</td>
<td>21.72 ± 2.96</td>
</tr>
<tr>
<td>Diabetic</td>
<td>15.68 ± 7.01</td>
<td>14.96 ± 3.30</td>
<td>18.22 ± 2.91</td>
<td>25.49 ± 1.71</td>
</tr>
<tr>
<td>Diabetic with insulin treatment</td>
<td>14.79 ± 4.36</td>
<td>14.20 ± 1.87</td>
<td>15.44 ± 2.59</td>
<td>20.33 ± 1.97</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 rats in each group. *P < 0.05 compared with nondiabetic controls.

Fig. 4. Phosphorylation of G6PD was increased in the kidney cortices of DM rats. A: protein kinase A (PKA) activity of NDM, DM, and INS animals. Data are means ± SE of 5 rats in each group. B: phosphoserine expression (top) and G6PD protein expression (bottom) (see methods for experimental protocols). C: summary of phosphoserine expression using densitometric analysis (see MATERIALS AND METHODS) normalized to G6PD protein level. D: correlation of phosphoserine expression to the PKA activity, which was calculated from values shown in C and A. E: correlation of G6PD activity to the phosphoserine expression, which was calculated from values shown in Figs. 2A and 4C. Correlation coefficient was calculated by linear regression analysis. *P < 0.05 vs. NDM group; †P < 0.05 vs. INS group.
may stimulate increased oxidative stress (41). Increased AGE causes increased oxidative stress (3, 14). Transforming growth factor-β, with a pattern similar to that reported for PKC and oxidative stress, can be activated by oxidative stress (2), and transforming growth factor-β can increase oxidative stress (39). In addition to the cultured cell experiments, studies with various diabetic animal models show evidence of increased oxidative stress in the target organs (31). Recently, Brownlee and colleagues (4, 19) proposed a unifying model that implicates oxidative stress as the precipitating cause of mechanisms responsible for diabetic complications. They have shown that mitochondria are a likely major source of oxygen radicals in cell culture and animal models of diabetes. In particular, they have shown that increased production of oxygen free radicals leads to inhibition of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase. Inhibition of this dehydrogenase would lead to an increase in the products of glycolysis that precede this step. According to their theory, increases in these products of glucose metabolism mediate the observed changes in at least four of the pathways that have been implicated as responsible for diabetic complications (increased aldose reductase, increased glucosamine, increased PKC activity, and increased AGEs) (4, 19). Thus there is very strong evidence that increased oxidative stress is of major importance in the etiology of diabetic complications.

The maintenance of the entire antioxidative system relies on sufficient NADPH production, as it is the principal cellular reductant. For example, NADPH is a critical cofactor for catalase in that it maintains the enzyme in an active state (12, 25, 26). Also, NADPH serves as a cofactor for glutathione reductase to which it catalyzes oxidized glutathione to its reduced form (GSH), a major free radical scavenger (Fig. 1).

As major source of NADPH, the role of G6PD as antioxidant enzyme has been recently well elaborated. For example, G6PD-null cells were reported to be exquisitely sensitive to oxidative stress (36). In addition, overexpression of G6PD helps to prevent oxidative stress-mediated apoptosis (30). It is notable that our previous data, as well data from others, suggested that G6PD was inhibited by high glucose significantly in cultured endothelial cells (1, 49). Data from the present study also showed that G6PD activity was significantly inhibited in the kidney cortices of the diabetic animals. It certainly is of interest to know whether the glucose-induced decrease in G6PD activity is observed in glomeruli, tubules, or both. Experiments in which the cortices were separated into glomerular and tubular fractions showed that both glomeruli and tubules displayed decreased G6PD activity in diabetic animals compared with NDM controls (data not shown). This observation is consistent with previously published data in which decreased G6PD activity has also been observed in liver (33), aorta (43), heart (27, 42), and Leydig cells (5) from diabetic animal models. Moreover, it has been reported that patients with diabetes have decreased G6PD levels in liver (6), mononuclear leukocytes (34, 44), and erythrocytes (9, 10). Our data, along with many others (13, 35, 47), strongly suggest that decreased G6PD activity is of significance in the pathogenesis of diabetic complications.

Most studies of oxidant stress and the antioxidant enzymes have focused on Cu,ZnSOD, MnSOD, catalase, and GPX and the GSH system. Often, G6PD activity and NADPH levels are not measured. There are intriguing studies in which G6PD was not measured; however, further interpretation of the data suggest that there may be a major underlying role for G6PD and NADPH in mediating the reported defects in the antioxidant system. For example, Ceriello et al. (7) published a report on skin fibroblasts from nondiabetic healthy people, nondiabetic people with kidney disease, people with Type 1 diabetes but no complications, and people with Type 1 diabetes and diabetic nephropathy. The skin fibroblasts were grown in either normal glucose (5.5 mM) or high glucose (22 mM). Cu,ZnSOD, MnSOD, catalase, and GPX were evaluated. The activities of all of the enzymes increased after exposure to high glucose in fibroblasts from the nondiabetic patients and from diabetic patients without complications. These enzyme increases would be expected as a response to increased oxidative stress caused by the high glucose. However, the fibroblasts from the diabetic patients with diabetic nephropathy showed no increases in MnSOD, catalase, or GPX. Only Cu,ZnSOD showed an increase. Although not reported in their paper, decreased activity of G6PD might be an underlying cause of decreased enzyme activities (or a contributing factor), as the NADPH from G6PD...
is a necessary substrate for GPX and a critical cofactor for optimal catalase activity. Recently, Hodgkinson et al. (22) studied peripheral mononuclear cells exposed to high glucose from control subjects, subjects with Type 1 diabetes and diabetic complications, and subjects with Type 1 diabetes and diabetic nephropathy. There was an increase in expression of catalase, Cu,ZnSOD, and GPX in the control group and the diabetic subjects without complications, but no increase was observed in the subjects with diabetic nephropathy. Aldose reductase inhibition restored the increased expression of the antioxidant enzymes in the mononuclear cells from the diabetic nephropathy group. As noted in their discussion, aldose reductase uses NADPH as a substrate; thus inhibition of aldose reductase leads to increased NADPH levels that may be the important mediator. As noted above for the Ceriello paper, decreased activity of G6PD might be the underlying or contributory cause for the changes observed in their experimental system.

The mechanism(s) responsible for the decrease of G6PD activity is not fully known. Our data suggest that both posttranslational modification (phosphorylation) and decreased expression play a role. It has been shown that high glucose leads to an increase of cAMP in various cell types, such as pancreatic islets (48), adipose tissues (23), bovine aortic endothelial cells (49), and human dermal microvascular endothelial cells (24). Moreover, our previous work from cultured endothelial cells showed that high glucose led to an inhibition of G6PD activity, at least partly due to the activation of cAMP-dependent PKA. Furthermore, inhibition of PKA by using the PKA inhibitor H-89, prevented the hyperglycemia-induced decrease in G6PD activity (49). In this study, we observed a significant increase in PKA activity in the kidneys from diabetic animals. These findings, along with the data showing increased phosphoserine phosphorylation of G6PD from diabetic animals suggest, although certainly does not prove, that PKA may be phosphorylating G6PD in vivo. However, considering the previously published in vitro data showing that PKA can directly phosphorylate G6PD and lead to inhibition of G6PD (49), it is quite possible that this action is occurring in vivo as well. Lastly, the mechanism for decreased expression of G6PD requires further study.

High glucose is the main determinant of the initiation and progression of diabetic nephropathy. Tight glucose control delays the onset and slows the progression of microvascular complications, including nephropathy in diabetic patients and diabetic animal models. Our results showed that administration of insulin reduced glucose concentrations, ameliorated oxidative stress, and normalized the activity of G6PD, thus restoring NADPH and GSH levels. It should be noted that insulin alone has been reported to increase G6PD activity (46). Therefore, whether the normalization of G6PD in diabetic animals is due to strict glucose control and/or to the impact of insulin on G6PD needs to be further clarified.

In conclusion, our data suggest that chronic hyperglycemia leads to a decrease of G6PD in kidney cortex, which leads to increased oxidative stress. This acquired G6PD inhibition in diabetic kidneys may partly be due to decreased G6PD expression and increased phosphorylation of G6PD caused by PKA activation.

ACKNOWLEDGMENTS

We are very grateful to Drs. Joseph Loscalzo and Jane Leopold of the Boston University Medical Center for assistance in the immunoprecipitation and Western blot experiments.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-54380 to R. C. Stanton and Juvenile Diabetes Research Foundation Grant 3-2004-573 to Y. Xu.

REFERENCES


