Effect of \textit{myo}-inositol on Cell Proliferation and Collagen Transcription and Secretion in Proximal Tubule Cells Cultured in Elevated Glucose\textsuperscript{1}

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\textbf{ABSTRACT}

Tubulointerstitial lesions often develop in the kidneys of patients and experimental animals with diabetes mellitus. In an in vitro model of diabetic renal disease, it has been previously demonstrated in this laboratory that elevated glucose levels stimulate procollagen transcription and secretion in proximal tubule cells in culture while inducing cellular hypertrophy and reducing cellular proliferation. Previous experiments in other tissues have suggested that \textit{myo}-inositol supplementation, probably by reversing a disturbance in cell \textit{myo}-inositol metabolism related to increased activity of the polyol pathway, reverses the effects of glucose on cell function. We tested the effect of \textit{myo}-inositol supplementation on proximal tubule cells in culture in the presence of elevated medium glucose level. Incubation in 450 mg/dL of glucose media reduced cell proliferation; 450 mg/dL of glucose plus \textit{myo}-inositol (800 \textmu M) increased proliferation, returning the value to that seen in cells incubated in 100 mg/dL of glucose. Incubation in 450 mg/dL of glucose media increased type IV and type I procollagen mRNA levels and peptide secretion rates compared with those seen in cells incubated in medium containing 100 mg/dL of glucose. This glucose-induced stimulation of procollagen mRNA levels and procollagen secretion was not observed when the elevated glucose me-

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\textbf{In diabetic nephropathy, renal failure is correlated with the development of interstitial fibrosis (1,2) as well as with the more widely recognized progressive mesangial expansion in the glomerulus (2). Renal fibrogenesis is a complex process involving the interaction of resident cells and circulating cells and the influence of local and systemic humoral factors. The contributions of the renal tubule epithelial cell and the renal fibroblast to this interstitial process in diabetes are unknown. Previously, by using a cell line derived from mouse cortical proximal tubules (MCT), we have found that high medium glucose inhibits cell proliferation, induces cell hypertrophy, and stimulates the transcription and secretion of basement membrane procollagen (type IV) and interstitial procollagen (type I) (3).}

Increased activity of the polyol pathway has now been shown to mediate many of the effects of high extracellular glucose levels through an alteration in cellular \textit{myo}-inositol metabolism (4). This, in turn, induces altered function in peripheral nerve (5), pigmented epithelium of the retina (6), and neovascular tissue (7) in early hyperglycemia. Winegrad et al. (4,8)
have postulated that in these tissues, reduction in a specific, labile cellular pool of myo-inositol, which is in equilibrium with extracellular myo-inositol, results from activation of the polyol pathway. Depending on the type of tissue, several disturbances in specific cellular function may then become manifest (4,8–11). In streptozotocin-induced diabetes in the rat, we have recently shown that the early glomerular hyperfiltration has also been prevented by feeding the animals a diet supplemented with myo-inositol (12).

In the current studies, we examined the effect of myo-inositol supplementation on the response of proximal tubule cells to high-glucose medium. Our results suggest that alterations in cell myo-inositol metabolism could underlie a number of the previously described effects of high ambient glucose on proximal tubule cells.

METHODS

Cell Culture

Details of cell isolation and characterization are as previously described (13). The cell line designated MCT ("mouse cortical tubule") is derived from microdissected proximal tubule segments from kidneys of normal SJL mice and is stabilized in long-term culture by virus transformation with a nonreplicating, noncapsid-forming strain of simian virus 40. Multiple morphological and functional criteria demonstrate that this cell line exhibits characteristics consistent with differentiated proximal tubule epithelial cells (13). MCT cell growth can be maintained in serum-free media for several days, and the cells elaborate many extracellular matrix proteins, predominantly laminin, procollagens type IV and V, and, to a lesser extent, procollagens type III and I.

Culture Media

The cell culture medium used is Dulbecco's modified Eagle medium (DMEM; GIBCO Laboratories, Grand Island, NY), containing a physiologically normal D-glucose concentration of 100 mg/dL, i.e., 5.5 mM, or a high D-glucose concentration of 450 mg/dL, i.e., 25 mM. The concentration of myo-inositol in this standard medium is 40 μM, close to that in blood of normal animals (5). The level of myo-inositol in the test medium is then varied between 40 and 1,200 μM. In all of the experiments in which DMEM is used, the media are supplemented with streptomycin (200 μg/mL), penicillin (192 μg/mL), glutamine (2 mM), and transferrin (1 μg/mL) (3).

The MCT cells are passaged every 48 to 72 h and are carried in DMEM supplemented with 10% inactivated fetal calf serum. The cells are incubated in a humidified atmosphere of 5% carbon dioxide at 37°C.

Thymidine Incorporation Assays

Thymidine incorporation is carried out by standard techniques as previously described (3). Cells are plated in 96 microtiter wells in serum-free DMEM. After 48 h, the media are removed and the cells are allowed to grow for an additional 48 or 72 h in various test media. The serum-free media tested contain 100 or 450 mg/dL D-glucose with variable concentrations of myo-inositol ranging between 40 and 1,200 μM. During the last 6 h of culture, the cells are pulsed with [3H]thymidine (5 Ci/mmol; Amersham Corp., Arlington Heights, IL) by the addition of 1 μCi per well. At the end of this period, the media are removed and the cells are released with trypsin-EDTA mixture (Flow Laboratories, Inc., McLean, VA) and are collected with a cell harvester (Brandel, Gaithersburg, MD) onto glass-microfiber filter paper (934-AH; Whatman, England). The radioactivity is assayed by counting filters in scintillation cocktail for β-emissions. [3H]thymidine incorporation into cell DNA is taken as an index of cell proliferation and is expressed in counts per minute per well. Each experimental condition is tested in three to six replicate wells, and the mean is taken to represent an individual experiment.

Radioimmunoassay of Procollagen Secretion

Details of the procedure are described elsewhere (3,13). MCT cells are plated in 24-well plates, and growth is rested in serum-free DMEM for 24 to 48 h. The media are then switched to fresh DMEM containing low- or high-glucose concentrations with or without myo-inositol supplementation. To measure procollagen secretion in the supernatants, the media are supplemented with 25 μg/mL of L-ascorbic acid and 25 μg/mL of β-aminopropionitrile, a cross-linking inhibitor. After another 48- or 72-h period, supernatants are removed from each plate, transferred into several U-bottom polystyrene chloride 96-microtiter wells (Costar, Cambridge, MA), and frozen at −20°C for subsequent solid-phase radioimmunoassay of soluble procollagens. Standard collagens type I and type IV are dispensed into polystyrene chloride wells at various concentrations for comparison with supernatants from MCT cells. The wells are dried overnight at 4°C, blocked with 4% BSA, and incubated with 1:100 dilution of affinity-purified polyclonal rabbit anti-type I and anti-type IV collagen antibodies (gifts of Dr. Antonio Martinez-Hernandez, Thomas Jefferson University, Philadelphia, PA). Binding of these primary antibodies is measured by using 125I-labeled anti-rabbit antibodies (10⁸ cpm/well) in 4% BSA. Binding of supernatants is compared with known standards as described previously (19).
Protein Synthesis

The methods for leucine incorporation and cellular protein content have been described previously (3). For leucine incorporation, cells cultured in 24-well plastic plates are used. After a 48-h quiescence, the cells are exposed for 48 h to various media and are pulsed during the last 14 h with 2 μCi/mL of [3H] leucine (70 Ci/mmol; Amersham). After the media are removed, the monolayers are washed with ice-cold phosphate-buffered saline (PBS), precipitated in 5% trichloroacetic acid for 10 min, and then washed with ice-cold deionized water to remove the aqueous phase of the label. The acid-precipitated monolayers are then solubilized with 0.5 N NaOH–0.1% Triton X-100 and counted for radioactivity. [3H]leucine incorporation is expressed per 10⁶ cells and is taken as an index of total protein synthetic rate. Cell number is determined simultaneously in replicate wells by using a Coulter cell counter.

mRNA Isolation and Measurement

The methods are essentially as described previously (3). For each experiment, 1 × 10⁶ to 1.6 × 10⁶ MCT cells are plated into each of four or more 75-cm² plastic flasks. After the cells are grown for 48 h in serum-free DMEM containing glucose (100 mg/dL), the media are replaced by fresh DMEM containing either 100 or 450 mg/dL of glucose. The final myo-inositol concentration is either 40 or 800 μM. After 72 h, the cells are released with a trypsin-EDTA mixture, washed twice in PBS, and counted. The cells are then lysed with 0.5% Nonidet P-40 in Tris-EDTA buffer. Cell equivalents (0.2 × 10⁶) of cytoplasmic RNA are denatured into a solution of 40% Formalin in 12x SSC (SSC is 0.15 M sodium chloride and 0.015 M sodium citrate, pH = 7.0) at 65°C for 15 min and then centrifuged, and the supernatants are spotted onto ZetaBind filters (CUNO Laboratory Products, Meriden, CN) by using a hybridomat manifold (Bethesda Research Laboratories, Inc., Gaithersburg, MD). The blots are warmed overnight at 42°C and are then prehybridized for at least 16 h in fluid containing 0.5 M phosphate buffer (pH = 7.2), 150 μg/mL of poly(A), 150 μg/mL of salmon sperm DNA, 7% sodium dodecyl sulfate, 1% BSA, and 1 mM EDTA at 42°C. The blots are then hybridized with 32P-labeled cDNA probes [SA, 0.5 × 10⁶ to 1.0 × 10⁹ cpm/μg] for 16 h at 45°C. The cDNA probes used are a 0.85-kilobase (kb) XhoI fragment containing the promoter-proximal exon encoding murine α5(1) procollagen from pAZ 1002 (14), a 0.66-kb PstI fragment encoding murine α1(IV) procollagen from pCS1 (15), and a 1.3-kb PstI fragment encoding rat glyceraldehyde phosphate dehydrogenase (GAPDH) from pBR322 (16). The probes are labeled with [32P]dCTP (>3,000 Ci/mmoll by random hexamer priming (17). The filters are washed 15 min twice in 3X SSC–0.5% sodium dodecyl sulfate at 55°C and then autoradiographed (Kodak XAR-5 film) with intensifying screens at −70°C for 1 to 3 days (GAPDH) or 10 to 20 days (procollagens). Relative mRNA levels are quantitated by densitometric scanning (Hoefer Instruments, San Carlos, CA) followed by area integration. Northern blot analysis demonstrated that MCT cells express the mature mRNA message for the cDNA probes used in this study.

Measurement of Cellular myo-Inositol Content

myo-Inositol levels are measured by a previously reported method (8). MCT cells are cultured as described for dot-blot mRNA experiments. After growth for 48 or 72 h, cell monolayers are washed three times with PBS and then the cells are lysed with ice-cold deionized water. A known amount of alpha-methyl mannose pyranoside is added to the flask before lysis as an internal standard for subsequent chromatographic assays. Cell lysates are centrifuged at 4°C, and the supernatants are collected and stored at −20°C for polyol measurements. Barium zine filters of lysed cells are prepared by the method of Somogyi (18) and lyophilized. The trimethylsilyl derivatives are extracted with hexane and injected on packed columns of 3% SE-30 on 80/100 GasChrom Q (Supelco) in a Hewlett-Packard 5890 gas chromatograph by using flame ionization detectors and a model 3392 integrator. Levels of myo-inositol are expressed in milligrams of cellular protein as determined by a modification of the Lowry assay.

Statistics

For each individual experiment, the mean of replicate determinations is calculated. The data are presented as means ± SE with N indicating the number of different experiments. Statistical analysis between two groups is performed by using paired or unpaired t test, as appropriate. Differences among several groups are tested by using analysis of variance. A value of P < 0.05 is considered to represent a significant difference.

RESULTS

[3H]thymidine Incorporation

Our previous studies showed that [3H]thymidine incorporation into DNA was significantly inhibited when medium glucose was elevated, and inhibition was most pronounced after a period of 48 or 72 h of exposing the cells to 450 mg/dL of glucose (3). In the current studies, the response to changes in myo-inositol content of the media was also assessed. In experiments not shown, we first demonstrated that myo-inositol supplementation increased thymidine

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incorporation only in media containing 450 mg/dL of glucose. This effect was dose dependent in the range between 40 and 1,200 µM. The effect at 800 µM was maximal with no further significant stimulation at higher myo-inositol levels. This concentration was therefore used in subsequent studies.

The results shown in Figure 1 summarize the findings in two different sets of experiments. Whether the test period is for 48 (Figure 1A) or 72 h (Figure 1B), the supplementation of the media with 800 µM myo-inositol leads to a restoration of [3H]thymidine incorporation in 450 mg/dL of glucose to values seen in 100 mg/dL of glucose media. Note that supplementation with myo-inositol of the media containing 100 mg/dL of glucose does not change cell proliferation significantly.

**Procollagen Secretion**

As seen in Figure 2, secretion of types IV and I procollagen is stimulated by 450 mg/dL of glucose media as we have previously found (3). When 800 µM myo-inositol is added to the media, there is a reduction in the high-glucose-induced stimulation of procollagen secretion. On the other hand, procollagen secretion in cells exposed to low-glucose medium is not significantly altered by myo-inositol supplementation of the growth medium.

**Type I Procollagen and Type IV Procollagen mRNA Isolation and Measurement**

We have previously shown by Northern hybridization that the mature mRNA for type I procollagen and type IV procollagen is expressed in MCT cells (unpublished observations) and that the effect of 450 mg/dL of glucose media to increase mRNA levels for type I procollagen and type IV procollagen is at least in part expressed at the level of gene transcription (3). The latter was concluded on the basis of the results of nuclear run-off assays which measure the rate of gene transcription. In the study presented here, we examined whether the effects of myo-inositol supplementation on procollagen secretion represented a change in message expression. To assess this, we measured steady-state mRNA levels. As shown in Figure 3, 450 mg/dL of glucose led to an increase in steady-state mRNA levels for type IV procollagen and type I procollagen, consistent with our previous findings (3). Again, myo-inositol supplementation reduced the levels of mRNA which are expressed in the cells exposed to the high glucose media. The results were similar in each of three different experiments (Figure 3). This response suggests an action of myo-inositol either on gene transcription or on the stabilization of mRNA levels. It is clear, however, that the myo-inositol-induced reduction in the increased
Proximal Tubule Cell Effects of myo-inositol

Figure 2. Effect of myo-inositol supplementation on procollagen secretion in proximal tubule cells. Cells were cultured for 48 or 72 h in media containing 100 or 450 mg/dL of glucose. Radioimmunoassay of procollagens was performed on conditioned supernatants, and data (mean ± SE) were expressed per 10^6 cells. When medium myo-inositol was 40 μM (open bars), procollagen secretion was significantly higher in the cells exposed to the high- versus the low-glucose concentration (*P < 0.05 for type IV procollagen, N = 7, panel A; *P < 0.05 for type I procollagen, N = 10, panel B). When medium myo-inositol was increased to 800 μM (shaded bars), procollagen secretion in the cells exposed to the high-glucose concentration was significantly reduced, to levels which were not different from those of cells exposed to the low-glucose concentration.

mRNA levels seen in the high-glucose media provide, at least in part, for the concomitant reduction in the glucose stimulation of procollagen secretion. Additional effects to modulate mRNA degradation were not addressed in this study. Levels of GAPDH mRNA were not altered by the concentrations of glucose or myo-inositol in the growth medium (data not shown).

Protein Synthesis

We previously determined that elevated glucose media stimulated protein synthesis in proximal tu-
bule cells (3). This, in part, may also account for the glucose-induced stimulation of procollagen synthesis and secretion. In this study, we tested whether the effect of myo-inositol supplementation to reduce glucose-induced procollagen secretion was reflective of a generalized effect on total cellular protein synthesis. The results shown in Figure 4 confirm our previous studies that elevated glucose levels stimulate [3H]leucine incorporation (3). However, myo-inositol supplementation has no effect on this response. We previously demonstrated that raising glucose concentration in the culture medium also increases the total protein content of proximal tubule cells (3). Along with the observed glucose-induced stimulation of leucine incorporation and the increase in cell size, as demonstrated by flow cytometry (3), the increase in total cellular protein content provided the evidence for glucose-induced cellular hypertrophy. In this study, we tested whether the hypertrophic response to glucose could also be prevented by myo-inositol supplementation. In nine different experiments, we found that raising the medium glucose from 100 to 450 mg/dL resulted in a 29 ± 10% (P < 0.025) increase in total cell protein content after 72 h, which confirmed our previous findings (3). However, when the high-glucose medium was supplemented with 800 μM myo-inositol, total protein content did not change significantly (210 ± 23 μg/10^6 cells in 800 μM myo-inositol versus 207 ± 29 μg/10^6 cells in 40 μM myo-inositol). Thus, the previously observed reduction in procollagen secretion induced by myo-inositol supplementation in the cells exposed to elevated glucose is not associated with a myo-inositol-induced inhibition in total protein synthesis or cellular protein content.

**Measurement of myo-Inositol Levels**

Raising medium glucose concentration from 100 to 450 mg/dL did not cause a decrease in MCT cellular myo-inositol content (Table 1) and in fact produced an increase. However, myo-inositol supplementation of the growth medium led to an approximately two-fold increase in myo-inositol content, both under conditions of physiologic (100 mg/dL) or elevated (450 mg/dL) medium glucose concentrations.

**DISCUSSION**

Tubulointerstitial damage and changes in the tubule cells of the diabetic kidney are prominent manifestations of the disease, but their significance and pathogenesis remain unclear (1–3,19). We have previously described a cell culture system using mouse proximal tubule epithelial cells where we tested the effects of high ambient glucose levels on growth and extracellular matrix biosynthesis (3). We found that raising media glucose concentrations to levels achieved in vivo in uncontrolled diabetes mellitus inhibited proximal tubule cell proliferation and increased cell size, total protein synthetic rate, and cell protein content. In addition, the biosynthesis of two representative procollagen components, type IV and type I, was also stimulated. Glucose-induced transcriptional control of procollagen genes was an important mechanism underlying this response. These data suggested that renal tubule epithelial cells could play a role in the formation of tubulointerstitial lesions in diabetic nephropathy.

In the series of studies presented here, we have extended the previous observations on the effects of high glucose on MCT cells and have examined the

![Figure 4. Absence of significant effect of medium myo-inositol supplementation on cell protein synthetic rate in proximal tubule cells. Cells were cultured for 48 h in serum-free media containing 100 or 450 mg/dL of glucose; myo-inositol concentration was 40 μM (open bars) or 800 μM (shaded bars). Cells exposed to the high-glucose concentration exhibited significantly increased [3H]leucine incorporation compared with cells exposed to the low-glucose concentration (P < 0.05), irrespective of the level of myo-inositol in the medium.](image)

**TABLE 1. Effect of myo-inositol supplementation on myo-inositol levels in proximal tubule cells**

<table>
<thead>
<tr>
<th>Glucose (mg/dL)</th>
<th>myo-Inositol in Media (μM)</th>
<th>myo-Inositol Content of Cells (μg/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>40</td>
<td>24.5 ± 0.3</td>
</tr>
<tr>
<td>100</td>
<td>800</td>
<td>42.1 ± 0.5</td>
</tr>
<tr>
<td>450</td>
<td>40</td>
<td>47.9 ± 0.5</td>
</tr>
<tr>
<td>450</td>
<td>800</td>
<td>90.2 ± 0.4</td>
</tr>
</tbody>
</table>

* Data are mean ± SE of cellular myo-inositol levels from three different experiments, each performed in duplicate. See Methods for details of gas chromatographic analysis.
Proximal Tubule Cell Effects of myo-inositol

hypothesis that the action of glucose could be the result of alterations in cellular myo-inositol metabolism, as has been reported to occur in other tissues exposed to high glucose levels (4). We found that elevating medium myo-inositol from 40 to 800 μM raised cell myo-inositol content and stimulated cell proliferation, reduced type I and type IV procollagen secretion, and normalized steady-state levels of mRNA for the two procollagen types. The effects on proliferation, procollagen secretion, and mRNA levels were specific for 450 mg/dL of glucose medium; no effect of myo-inositol supplementation was observed in 100 mg/dL of glucose media.

It should be noted, however, that not all of the effects of high glucose can be normalized by myo-inositol supplementation. For instance, the glucose-induced stimulation of total protein synthesis and of cellular protein content was not inhibited by myo-inositol supplementation. This finding also provides for two conclusions. First, the effect of myo-inositol on procollagen transcription and secretion is not a nonspecific effect. Second, an absence of an effect of myo-inositol on total protein synthesis and cellular protein content may also imply that the cellular hypertrophy observed in the cells exposed to high glucose levels (3) is not reversed by myo-inositol supplementation. Because myo-inositol supplementation leads to a dissociation of glucose-induced cell hypertrophy from increased extracellular matrix synthesis, these results also suggest that different cellular mechanisms may underlie these two effects of high-glucose medium on proximal tubule cells.

Glucose-induced inhibition of cellular proliferation has been previously described for cultured bovine retinal pericytes (20) and human endothelial cells (21). The underlying mechanism for glucose-induced inhibition of DNA synthesis remains to be elucidated but may relate to alterations in cell-cycle traversal which have been previously described in cultured endothelial cells (22) and which we have also confirmed in MCT cells (unpublished data). Proximal tubule cells (23) and endothelial cells (24) share the property that glucose uptake readily occurs without the requirement for insulin. Thus, increased activity of the polyol pathway and subsequent abnormalities in myo-inositol metabolism could be an important response to high-glucose medium in these tissues. Although this pathway has been well described in endothelial cells (25), the presence of this system in proximal tubule cells has not been described until very recently. We, with MCT cells (26), and others, with a cell line derived from human proximal tubule cells (27), have shown the induction of sorbitol formation in these two cell systems when incubated in high-glucose media. Thus, the effect which we observed with myo-inositol supplementation may represent an action similar to that seen in endothelial cells and nerve cells exposed to high glucose levels where increased extracellular myo-inositol specifically reverses a cellular defect which results from polyol pathway activation in those tissues (4,28).

The interaction between medium glucose concentration and myo-inositol in modulating cell growth and proliferation in other cell culture systems has been evaluated by several groups with varying results, depending on the cell type examined. Endothelial cells show an absolute requirement for at least 16 μM myo-inositol for proliferation and demonstrate maximum response at 160 μM myo-inositol (21). In that system, myo-inositol supplementation was found to enhance cell proliferation in the presence of elevated medium glucose but the response was not complete and the inhibitory action of glucose persisted (21). Yorek et al. have also studied the effects of high glucose on endothelial cells (29) and neuroblastoma cells in culture (25). In neuroblastoma cells, the content of myo-inositol was depressed after a 2-wk incubation in high-glucose medium, primarily the result of a noncompetitive inhibition in the uptake of myo-inositol (25). In endothelial cells, there was also a reduction in cellular myo-inositol levels after prolonged exposure to elevated glucose levels but, in that system, the inhibition was competitive (29). However, in these two cell systems, unlike those in other reports noted above (21,22), a clear inhibitory action of glucose on cell number was not found. Thus, the results we observed on cell proliferation and the response to myo-inositol supplementation in MCT cells may be applicable to some, but not all, cell culture systems.

A characteristic feature of the diabetic state is the increased thickness of basement membranes such as those lining the retinal and glomerular capillaries as well as renal tubule epithelia (29–34). We have previously shown that proximal tubule cells exposed to high glucose levels secrete approximately twice as much procollagen type IV as do cells exposed to normal glucose concentration, and this response could be accounted for, at least in part, by increased biosynthesis of procollagen molecules (3). Similar increases in collagen secretion have been described for retinal pericytes (20) and endothelial cells (35) cultured in high-glucose medium. In this study, we demonstrate that myo-inositol supplementation of elevated glucose media reduces the increased procollagen mRNA levels and the peptide secretory rates.

The current study also demonstrated that myo-inositol supplementation reduced the glucose-induced stimulation of mRNA levels and secretion rates of procollagen type I, an interstitial-type extracellular matrix component. Interstitial fibrosis is a prominent feature of established diabetic nephropathy (1,2). On the basis of studies of kidney biopsies from patients with diabetic nephropathy, there is a close correla-
tion between the degree of tubulointerstitial fibrosis and mesangial expansion (1,2). Fibrogenesis in the kidney is complex and involves the interaction of resident cells with local and systemic humoral factors (19). The proximal tubule cell is a likely participant in the fibrogenic response of diabetic renal disease. Proximal tubule cells are derived from mesenchyme (36) and retain the capability of producing finite, although limited, amounts of interstitial-type matrix components (13,19). Even small increases in secretory rates of interstitial collagens elaborated by each cell could eventually contribute to the cumulative fibrosis as proximal tubule cells constitute the bulk of renal cortical volume.

The current results suggest that the alterations in procollagen secretion and cellular proliferation may be linked to a putative defect in myo-inositol metabolism induced by elevated extracellular glucose. The absence of an action of myo-inositol in 100 mg/dL of glucose supports this hypothesis. These results, by analogy with those obtained in other systems such as diabetic nerve and vascular smooth muscle, suggest that altered myo-inositol metabolism is a central mechanism whereby elevated glucose leads to a functional disturbance in various cell types (4).

The observation that elevated extracellular fluid glucose concentrations may cause alterations in cellular function correctable by myo-inositol supplementation of the medium, but without producing a depletion in the cellular myo-inositol content, is not surprising. Glomerular hyperfiltration reversible by dietary myo-inositol supplementation is demonstrable in streptozotocin-diabetic rats (12) weeks before there is any detectable decrease in tissue myo-inositol content (37). Furthermore, in intact preparations of arterial wall isolated from normal rabbits, incubations in medium containing elevated glucose concentrations result in functional alterations which are completely reversible by myo-inositol supplementation of the medium and occur in the absence of any change in composite tissue myo-inositol content (11). The mechanism appears to be depletion of a small, discrete pool of myo-inositol which is in equilibrium with extracellular myo-inositol and which is required for the synthesis of a specific pool of phosphatidylinositol, whose turnover regulates a distinct component of Na⁺,K⁺-ATPase activity, probably through activation of a specific protein kinase C (11). Although a global decrease in polyphosphoinositide content or metabolism has not been reported as part of the acute tissue response to diabetes, secondary increases in polyphosphoinositide metabolism have been reported in diabetic nerve (38) and increased responsiveness to agonists whose signal-transduction mechanism is via a polyphosphoinositide hydrolysis have been reported in human (39) and experimental diabetes (40).

The cellular mechanisms whereby the change in extracellular myo-inositol levels result in a change in procollagen secretion and in procollagen mRNA levels were not studied in these experiments. Important changes, particularly in protein kinase C activity, have been a feature of several systems exposed to high extracellular glucose levels, and such actions may also provide a link explaining the mechanism of changes in gene activation resulting from altered glucose and myo-inositol levels (28,41).

In conclusion, these data extend our previous studies demonstrating the effects of glucose to induce cellular hypertrophy, inhibit cell proliferation, and stimulate procollagen types I and IV biosynthesis in proximal tubule cells. They also provide evidence for a role of altered cellular myo-inositol metabolism in the response to high ambient glucose levels (12,28,41), as myo-inositol supplementation corrects some of these glucose-associated abnormalities (namely inhibition of cell proliferation and increased procollagen gene transcripts and peptide secretion). Because myo-inositol supplementation leads to a dissociation of glucose-induced cellular hypertrophy from increased extracellular matrix biosynthesis, our findings also suggest that distinct cellular mechanisms may underlie these two effects of high medium glucose levels on proximal tubule cells. The mechanisms of these effects and the potential implications for the pathogenesis and therapy of diabetic nephropathy remain to be understood.

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