Biochimica et Biophysica Acta, 1138 (1992) 229–235

BBADIS 61125

Unimpaired formation of hormone-stimulated inositol trisphosphate in human mesangial cells under hyperglycemic conditions

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(Received 24 July 1991)

Key words: Phosphatidylinositol; Inositol trisphosphate; Myo-inositol; Bradykinin; Vasopressin; Mesangial cell; (Kidney)

The relationship between bulk cellular *myo*-inositol content and phosphatidylinositol metabolism was evaluated in a human mesangial cell line under euglycemic and hyperglycemic conditions. Mesangial cells maintained in high glucose medium displayed a concentration-dependent fall in *myo*-inositol as measured by gas-liquid chromatography. Measurements of phosphatidylinositol, phosphatidylinositol 4-monophosphate and phosphatidylinositol 4,5-bisphosphate mass revealed slight but statistically insignificant increases in cells exposed to high glucose containing medium. CDP-diacylglycerol: *myo*-inositol 3-phosphatidylinositol transferase activity, measured in plasma membranes from mesangial cells grown under control and hyperglycemic conditions, was kinetically similar with Michaelis constants (K_m values) for *myo*-inositol of 2.9 and 2.1 mM, respectively. Finally, hormone-stimulated intracellular calcium mobilization and *myo*-inositol 1,4,5-trisphosphate mass was measured from mesangial cells grown under normal and hyperglycemic conditions. Both intracellular calcium and inositol trisphosphate formation were unchanged in cells previously exposed to high glucose conditions (400 mg/dl) compared to cells grown under normal glucose concentration (100 mg/dl). These data indicate that bulk changes in *myo*-inositol induced by hyperglycemia are neither associated with alterations in basal levels of inositol containing glycerolipids nor with changes in hormone-stimulated calcium mobilization and inositol trisphosphate formation under conditions of short term changes in extracellular glucose.

Introduction

The association between *myo*-inositol metabolism and diabetic nephropathy remains controversial. Observed decreases in *myo*-inositol content under hyperglycemic conditions have led to the postulate that diabetic injury is mediated in part by derangements in hormone-stimulated PIP2 hydrolysis [1,2]. Support for this hypothesis based on the association of functional derangements such as Na^+/K^+-ATPase activity and glomerular filtration with *myo*-inositol depletion and the ability of *myo*-inositol supplementation to reverse these derangements [3].

Biochemical support for this hypothesis, however, has been inconsistent. Levels of *myo*-inositol [4], inositol glycerolipids [5,6], protein kinase C activity [7,8] and Na^+/K^+-ATPase activity [6,9] are reportedly unchanged, low or elevated depending on the tissue studied and the experimental conditions employed. In addition, previous reports in a kidney cell line have suggested that bulk *myo*-inositol does not regulate inositol phosphate formation under hyperosmolar conditions [10]. The 'polyol hypothesis' has not been tested by direct measurement of hormone-stimulated inositol 1,4,5-trisphosphate (InsP3) mass. Such a test is critical because reliance on cellular incorporation of [^{3}H]inositol may be misleading since the specific activity of radiolabelled inositol phosphates may differ under euglycemic versus hyperglycemic conditions independent of changes in the chemical mass of these products.

We studied the metabolism of inositol containing glycerolipids in a human mesangial cell line to evaluate the association between cellular *myo*-inositol content and InsP3 formation under short term hyperglycemic conditions. We report that *myo*-inositol content falls when mesangial cells are grown in high glucose

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medium. This fall is unassociated with changes in inositol glycerolipid content, hormone-stimulated InsP3 mass, and intracellular calcium mobilization.

Research design and Methods

Materials. High performance TLC 60 plates were from E. Merck (Montreal, Canada). Phospholipid standards including CDP-diglyceride were from Sigma (St.Louis, MO) or Serdary (Port Huron, MI). Tri-octylamine was from Aldrich Chemical (Milwaukee, WI). Freon was from Matheson gas products (East Rutherford, NJ). *Myo*-[2-^{3}H]inositol containing radioisotopes and aqueous liquid scintillant were from Amersham (Arlington Heights, IL). L-*chiro*-inositol standard was the generous gift of Dr. B.W. Agranoff (University of Michigan, Ann Arbor, MI). Tri-Sil Concentrate and Reacti-vials (0.3 ml) were from Pierce Chemical (Rockford, IL). Cell culture media and sera was obtained from Gibco (Grand Island, NY) and Sigma (St. Louis, MO).

Mesangial cell culture, characterization and incubation. Human mesangial cells were obtained from the laboratory of Dr. David Lovett (University of California, San Francisco). These cells maintain a phenotype which is histologically similar to rat mesangial cells. They are positive for actin, desmin and vimentin and negative for cytokeratin by immunocytochemical staining. These cells also form hillocks following several days in culture. Cells were cultured in a medium consisting of Waymouth's medium and 20% fetal calf serum. Cells were studied between the fifth and tenth passage and continually replenished from frozen stock. Prior to biochemical measurements, mesangial cells were rendered quiescent by changing the medium to Dulbecco's modified Eagles medium without serum. This media contained glucose at a concentration of 100 mg/dl and was supplemented to obtain the desired glucose concentration.

Myo-inositol determination. Following exposure to media of differing glucose content, mesangial cells were scraped, homogenized in 6% trichloroacetic acid and allowed to stand on ice for 5 min. 5 nmol/dish of L-*chiro*-inositol was added as an internal standard. The suspension was subsequently centrifuged at $30 \times g$ for 5 min at 40°C. The pellet was saved for protein determination and the supernatant was removed and vortexed with a solution of 26% tri-octylamine and Freon to remove the trichloroacetic acid [11]. The suspension was again vortexed and centrifuged at $270 \times g$ for 10 min at 4°C to permit accelerated phase separation. The upper aqueous phase was used for derivatization and *myo*-inositol mass measurement.

An adaptation of the method described by Rittenhouse [12,13] was employed. 50 μl aliquots of tissue extracts obtained as above were applied to clean and dry reacti-vials. An equal volume of GC-grade pyridine was also added, vials were capped, vortexed and dried under a stream of nitrogen. Two other rinses of pyridine were performed to assure complete removal of water. To the dried samples 25 μl of dry pyridine was added, vials capped, vortexed and heated at 70°C for 15 min. This was followed by the addition of 25 μl of a Tri-Sil : pyridine mixture (3 : 10) to the still-warm samples which again were vortex-mixed and heated for 20 min at 70°C. the samples were dried under N_2 flow and resuspended in HPLC grade acetonitrile for GC injection.

Separation and detection of the trimethylsilyl derivatives of *myo*-inositol and L-*chiro*-inositol were performed on a Hewlett-Packard 5890 chromatograph with an SP-2380 capillary column (Supelco, Bellefonte, PA) Split injection (33 : 1) and flame ionization detection were employed. Nitrogen was used as the carrier gas at a flow rate of 2.5 ml/min. Injector and detector temperatures were 200°C, column temperature was 100°C and ramped at 3.5°C/min. Under these conditions L-*chiro*- and *myo*-inositol eluted at 17.3 and 20.7 min, respectively. *Myo*-inositol mass was calculated from the ratio of *myo*-inositol and L-*chiro*-inositol peak areas corrected for their respective response factors. The signal to noise ratio was always greater than 10 : 1 and potential interference by reagent and tissue blanks was ruled out.

Intracellular space measurements were conducted as described by Pollock [14]. 3-*O*-[methyl-^{3}H]D-glucose was utilized as the intracellular marker; [^{14}C]inulin was utilized for the extracellular space marker.

Phospholipid extraction, separation and identification. Prior to lipid extraction an aliquot consisting of 5% of each fraction was removed for protein determination utilizing fluorescamine [15]. Bovine serum albumin was used as standard. 5 nCi of individual tritiated inositol lipid standard was added to each sample to monitor recovery. Lipids were extracted by the addition of chloroform : methanol : 1 M HCl (10 : 20 : 10) to isolated scraped cells. The mixture was vortexed and centrifuged at $270 \times g$ for 10 min at 4°C to affect phase separation. The upper aqueous phase was removed, the protein pellet at the interface deflected, and the lower organic phase recovered. Both upper and lower phases were back extracted and the lower phases were pooled and evaporated to dryness under a stream of nitrogen. The lipid extract was resuspended in 50 μl of chloroform:methanol (1 : 1).

Extracted phospholipids and phospholipid standards were separated on high-performance thin-layer chromatography plates. Phosphatidylinositol 4-monophosphate and phosphatidylinositol 4,5-bisphosphate were separated by the method of Jolles [16]. Phosphatidylinositol separations were conducted one-dimensionally in a solvent system consisting of chloroform: methanol:

40% methylamine (63:35:10). Phospholipids were identified both by autoradiography and by comparison to authentic standards. For routine analysis plates were exposed to iodine vapor, and phospholipids scraped and quantitated by the method of Vaskovsky [17]. All values were corrected for recovery of radioactivity.

Phosphatidylinositol synthase assay. Synthase activity was assayed following the protocol of Imai and Gershengorn [18,19]. Isolated plasma membranes were suspended in 50 μl of buffer containing Tris-HCl 100 mM (pH 7.5), EGTA 1 mM, $MgCl_2$ 3 mM, $MnCl_2$ 3 mM, 0.1% Triton-X 100, 0.8 mM CDP-dipalmitoyl glycerol and 0.1 to 10 mM *myo*-inositol containing 10^6 dpm/nmol of *myo*[2-^{3}H] inositol. The incubation proceeded for 10 min at 37°C and was terminated by the addition of 1.5 ml of chloroform : methanol : concentrated HCl (100 : 100 : 1). Phase separation was facilitated by the addition of 0.375 ml of 10 mM EDTA. The lower phase was removed and the upper phase was back extracted with 1.5 ml of the same organic phase. The lower phases were then combined, evaporated to dryness under nitrogen and the [^{3}H]phosphatidylinositol formed was separated by high performance TLC as above. Phosphatidylinositol was identified by co-migration with authentic standards and quantitated by liquid scintillation counting.

Intracellular calcium determination. Mesangial cells were grown from 24 h in serum-free Dulbecco's medium containing either 100 or 400 mg/dl of glucose. The cells were then trypsinized and loaded with 2 μM Fura-2 AM for 20 min. After loading the cells were washed twice with phosphate-buffered saline and resuspended at a final concentration of 10^6 cells per ml. 2 ml of cell suspension was loaded into a quartz thermostated cuvette. Fluorescence was determined at excitation wavelengths of 340 and 380 nm and an emission wavelength of 505 nm in a Spex spectrofluorometer. Cells were stimulated with agonists at concentrations of 1 μM, and calibrations were performed with 4 mM EGTA and 50 μM digitonin. Calculations were performed according to the method of Grynkiewicz [20].

Inositol 1,4,5 trisphosphate determination. InsP3 was measured by a radioreceptor binding assay utilizing calf adrenocortical microsomes following the method of Palmer et al. [21] as recently applied to MDCK cells [10]. In brief, binding protein was prepared from adrenal glands obtained from freshly slaughtered calves. The cortex was dissected free and the tissue homogenized in 20 mM $NaHCO_3$ and 1 mM dithiothreitol. The homogenate was then centrifuged at 5000 $\times g$ for 15 min. The supernatant was collected and centrifuged at 35000 $\times g$ for 20 min. The pellet was resuspended in the homogenizing buffer and recentrifuged at 35000 $\times g$ for 20 min. Binding protein was stored at a concentration of 20 mg/ml at −80°C.

InsP3 was assayed in an incubation buffer consisting of 100 mM Tris buffer (pH 8), and 4 mM EDTA. Incubations were performed for 15 min on ice with a mixture consisting of 0.6 mg binding protein, 1 nCi of *myo*-[2-^{3}H]inositol 1,4,5-trisphosphate (specific activity 40–60 Ci/ mmol) and cellular inositol phosphate extract or inositol phosphate standard in a final vol. of 120 μl. Incubations were terminated by centrifugation at 16000 $\times g$ for 4 min. The supernatant was then removed. Specific binding for microsomal membranes used within 2 months of preparation was in excess of 25% of the total radioactivity. Nonspecific binding ranged from 10 to 20% of the protein associated activity. To rule out possible dephosphorylation or phosphorylation of the radiolabeled *myo*-inositol trisphosphate, 5 nCi of *myo*-[2-^{3}H]inositol 1,4,5 trisphosphate were incubated with binding protein for 1 h at room temperature. The mixture was treated with trichloroacetic acid and subjected to chromatographic analysis as previously described [22]. A single peak of activity cochromatographing with authentic Ins1,4,5P3 was observed with no conversion to inositol or inositol mono-, bis-, tris- or tetrakisphosphates.

Results

Fig. 1 displays the *myo*-inositol content of mesangial cells in response to incubation in varying concentrations of glucose for 24 h. Statistically significant changes in *myo*-inositol content were observed at all concentrations of glucose greater than 100 mg/dl. The effects of osmolality were assessed by incubating cells in the presence of mannitol in a concentration sufficient to

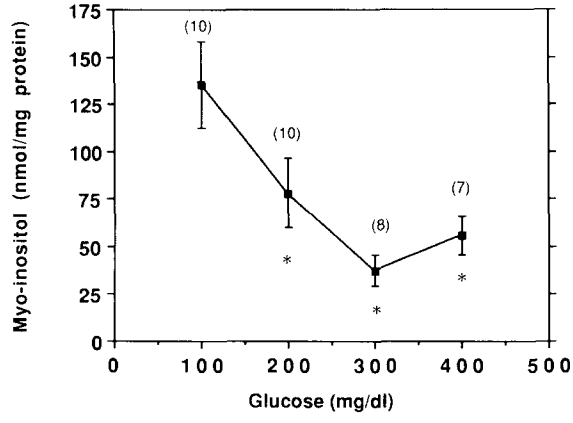

Fig. 1. *Myo*-inositol content of human mesangial cells grown for 24 h in varying concentrations of glucose. Cells were plated and grown in Waymouth's medium with 20% fetal calf serum for 48 h. The media was then changed to Dulbecco's minimal essential medium containing varying glucose concentrations as indicated. *Myo*-inositol was measured by gas-liquid chromatography as described in Research design and Methods. Data are expressed as nmol *myo*-inositol per mg cell protein. * Denotes $P < 0.01$ by unpaired t analysis versus the 100 mg/dl condition.

TABLE I

Chemical measurements of inositol glycerolipids in mesangial cells exposed to 100 or 400 mg/dl glucose for 24 h

Values represent nmol lipid/mg protein and are expressed as the mean ± S.E. for eight determinations.

	100 mg/dl	400 mg/dl
Phosphatidylinositol	42.4 ± 1.5	45.7 ± 1.9
Phosphatidylinositol 4-monophosphate	3.37 ± 1.04	4.98 ± 0.63
Phosphatidylinositol 4,5-bisphosphate	4.95 ± 1.37	8.49 ± 1.46

bring the initial medium osmolality to that equal to 400 mg/dl of glucose. Under these conditions cellular *myo*-inositol levels were substantially greater than in cells incubated with 100 mg/dl glucose alone (data not shown). These data are consistent with the interpretation that the effects of hyperglycemia on *myo*-inositol levels were unrelated to simple changes in extracellular osmolality.

Cell volume measurements were conducted to provide estimates of total cellular inositol concentrations. Cell vols. were 7.9 μl/mg protein under normoglycemic conditions and 6.9 μl/mg protein following incubation with 400 mg/dl glucose for 24 h. Total cellular *myo*-inositol concentrations would therefore be 17.1 and 5.4 mM, respectively.

The inositol glycerolipid content was determined in cells grown in media containing 100 or 400 mg/dl glucose for 24 h. The results are displayed in Table I. Measured levels of phosphatidylinositol, phosphatidylinositol mono- and bisphosphate were slightly greater in mesangial cells exposed to high glucose media. Although the inositol glycerolipid levels were consistently

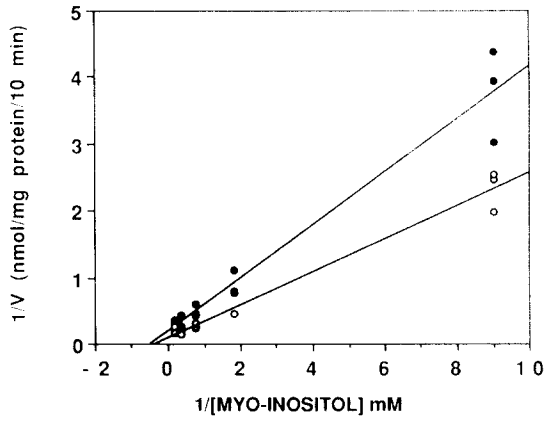

Fig. 2. Substrate (mM *myo*-inositol) versus velocity (nmol phosphatidylinositol per mg protein per 10 min) profile of the phosphatidylinositol synthetase activity from isolated mesangial cell membranes. Synthetase activity was assayed from cells incubated for 24 h in medium containing 100 mg/dl of glucose (closed circles) or 400 mg/dl of glucose (open circles).

greater under hyperglycemic conditions, these changes were not statistically significant.

CDP-diacylglycerol : *myo*-inositol 3-phosphatidylinositol transferase (PI synthase) activity was measured in mesangial cell membranes grown at 100 and 400 mg/dl for 24 h. Fig. 2 displays the substrate versus velocity profile under conditions of varying *myo*-inositol concentration. The apparent K_m values for *myo*-inositol were 2.9 and 2.1 mM for membranes obtained from cells grown at 100 and 400 mg/dl, respectively. The V_{max} of the synthetase from mesangial cells grown at high glucose conditions was slightly greater than that of cells grown under normal glucose conditions.

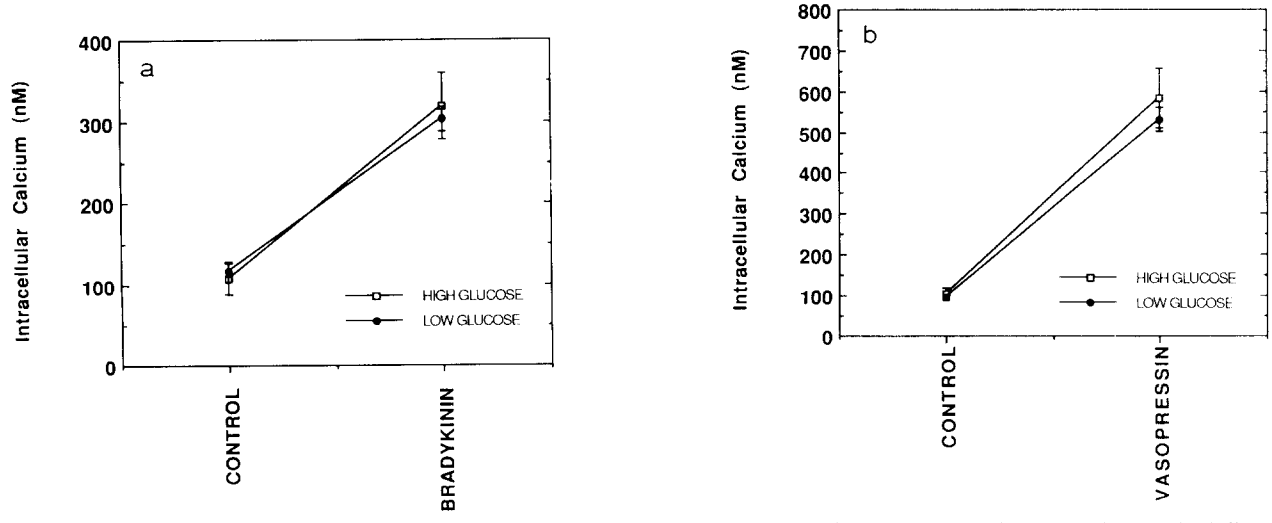

Fig. 3. Intracellular calcium concentration in mesangial cells grown in low glucose (100 mg/dl) or high glucose (400 mg/dl) for 24 h. Cells were exposed to 10–6 M bradykinin (A) or vasopressin (B). The data represent the mean ± S.E. of eight independent measurements. Differences between high and low glucose conditions were not significant by the unpaired *t* test.

TABLE II

Hormone-stimulated inositol 1,4,5-trisphosphate

InSP3 measurements in mesangial cells grown in high or low glucose. Cells were exposed to high or low glucose-containing media for 24 h and then extracted for InsP3 determination or stimulated for 15 s with either bradykinin or arginine vasopressin. The data represent the mean ± S.E. of six determinations. No significant differences were observed between high and low glucose groups by paired *t* analysis.

	100 mg/dl	400 mg/dl
Control	6.18 ± 1.63	7.31 ± 2.21
Bradykinin	15.5 ± 1.41	16.9 ± 2.7
Arginine vasopressin	14.9 ± 2.04	17.9 ± 3.54

Attempts to measure InsP3 formation in intact mesangial cells following stimulation with angiotensin II were unsucessful due to the loss of angiotensin II responsiveness after four to five cell passages. Hormone responsiveness to bradykinin and arginine vasopressin, however, persisted after multiple cell passages. When cells were preincubated for 24 h to high or low glucose concentrations and then exposed to either bradykinin or arginine vasopressin, no differences in intracellular calcium mobilization were evident when expressed as the peak calcium transient (Fig. 3a and b). Calcium transients were no different at 10^{-6} M. Parallel measurements of InsP3 demonstrated no glucose dependent changes under basal or hormone-stimulated conditions (Table II).

Discussion

The glomerulus and more specifically the mesangium is regarded as a target for injury associated with diabetic nephropathy [23]. The mesangial expansion observed in diabetes may result from the altered metabolism of macromolecules by mesangial cells, and the increase in glomerular filtration observed in early diabetes may result, in part, from altered contractility of mesangial cells. Observed changes in *myo*-inositol content in diabetic rat glomeruli [24] and the correction of hyperfiltration by *myo*-inositol supplementation have been cited as supporting a role for polyols, including sorbitol and inositol, in the mediation of diabetic nephropathy [3,25,26].

Proponents of the 'polyol' hypothesis of diabetic injury have argued that decrements in *myo*-inositol content serve as the basis for many of the derangements seen in diabetic injury. Four testable predictions are implicit in this hypothesis. The first prediction is that *myo*-inositol is rate limiting for the synthesis of inositol-containing glycerolipids. The second prediction is that levels of inositol containing glycerolipids should reflect changes in *myo*-inositol. The third prediction is that the products of PIP2 hydrolysis, InsP3 and diglyceride, should decrease as a result of impaired substrate formation. The final prediction is that direct functional responses which are InsP3 dependent, viz. intracellular calcium mobilization, should be impaired.

With regard to the first prediction, phosphatidylinositol is formed by the enzymatic association of *myo*-inositol and CDP-diglyceride. The enzyme responsible for phosphatidylinositol formation, phosphatidylinositol synthetase, has recently been characterized in both rabbit proximal tubule cells [27] and in Madin Darby canine kidney cells [10]. The proximal tubule cell activity can be detected in brushborder and basolateral membrane fractions as well as in microsomal membranes. The rabbit kidney synthetase displays an apparent Michaelis constant (K_m) for *myo*-inositol of 0.2 to 0.3 mM, and the enzyme activity is inhibited by phosphatidylinositol. The K_m of the phosphatidylinositol synthetase of MDCK cells is comparably low (0.9 to 1.0 mM), and it is unchanged under hyperosmolar conditions where cellular *myo*-inositol content increases 3-fold [10]. In both tissues the estimated cellular *myo*-inositol concentrations are significantly greater than those required to fully saturate the synthase. Aside from the fact that cellular *myo*-inositol concentrations are greater than the apparent K_m for the synthase, another property of this enzyme may account for the stability of PI levels within cells. The activity of the synthase is regulated by the level of its own product. Although synthase activity may be detected in non-microsomal sites, there is no evidence that the synthase exhibits kinetically dissimilar properties in one site versus another.

Reported data supporting an association between hyperglycemia and decreased levels of inositol lipids are inconsistent. Palmano et al. reported a decrease in lipid inositol in sciatic nerve in acutely diabetic rats. However, tissue inositol levels were unchanged from control animals at this point in time. Furthermore, brain, kidney and liver inositol lipid levels were unchanged under almost every condition in which inositol content was decreased [5]. Under conditions where *myo*-inositol is reported to regulate inositol lipid formation, investigators have often relied upon $^{32}PO_4$ incorporation. For example, decreased labelling of PIP and PIP2 has been reported in glomeruli from diabetic rats acutely exposed to *myo*-inositol [7]. However, the radiolabelled precursors for inositol lipid formation (PI, PIP, PIP2, ATP, CTP and CDP-diglyceride) may have markedly different specific activities under the experimental conditions employed.

For similar reasons, assessing InsP3 formation with radiolabelling under conditions of hyperglycemia is potentially misleading. The utilization of direct measurements of InsP3 mass eliminates potential artifacts associated with variable levels of radiolabel incorporation.

The other product of PIP2 hydrolysis, diglyceride, may arise from alternative pathways including hydrolysis of phosphatidylcholine, sphingomyelin synthesis or de novo synthesis from glucose.

Supporting data for hypotheses linking cellular *myo*-inositol and diabetic injury have been obtained primarily from diabetic nerve and are based on the association of bulk *myo*-inositol content and functional derangements such as Na^+/K^+-ATPase activity and conduction velocity [27]. Biochemical support for these associations, however, has been inconsistent. Levels of inositol-containing glycerolipids, protein kinase C activity and Na^+/K^+-ATPase activity are reportedly unchanged, low or elevated. Understanding the biochemical basis for these changes is problematic when glomerular preparations are employed in part due to the cellular heterogeneity. In addition, the glomerular hypertrophy associated with diabetes makes the comparison of metabolite concentrations more difficult to interpret. The use of cell culture models offers a less heterogeneous model, but may provide a less accurate model of the diabetic state. On the one hand, the cell line utilized in the present study requires high glucose for long term maintainence and does not respond to angiotensin II after serial passages. In this respect these cells are similar to primary cultures of rat mesangial cells. On the other hand, potential confounding variables in studies utilizing tissues from diabetic animals, such as the independent role of glucoregegulatory hormones, are avoided with in vitro culture models.

The association between bulk *myo*-inositol content, calcium mobilization and Ins P3 formation in mesangial cells was evaluated in the current study. Exposure of cells to medium of varying glucose concentrations was associated with a concentration-dependent fall in total cellular *myo*-inositol. Incubation of mesangial cells with mannitol increased cellular *myo*-inositol content. The interpretation of these data is consistent with the competitive inhibition of *myo*-inositol uptake by glucose as previously studied in rat mesangial cells [28]. Mannitol which apparently does not compete with *myo*-inositol at the cotransporter creates an osmotic challenge resulting in increased cellular *myo*-inositol.

Despite the significance of this fall, inositol glycerolipid levels were unchanged under high glucose conditions. Phosphatidylinositol synthase activity was kinetically similar when measured under euglycemic and hyperglycemic conditions. The apparent K_m values for inositol were between 2 and 3 mM. Although these values were approx. 10-fold greater than those observed in both proximal tubule cells and 2-fold greater than in MDCK cells, the calculated bulk cellular inositol concentrations were 5.4 to 17.1 mM, more than 2-times that required to saturate fully the synthetase. Of note was the observation that the *myo*-inositol content expressed as nmol/mg protein was greater than that recently reported for rat mesangial cells in culture [28]. Whether this variance reflects differences in experimental conditions or species variability will require further investigation.

Several authors have argued for the existence of hormone-sensitive pools of *myo*-inositol and have suggested that derangements in these pools account for alterations in phosphatidylinositol turnover in the absence of changes in total inositol-containing glycerolipids. Aortic endothelial cells [29] and a mammary cell line, WRK-1 cells [30,31] have been used as models to evaluate this hypothesis. In the former case these investigators reported that basal but not stimulated PI turnover was affected by *myo*-inositol content and that this regulated Na^+/K^+-ATPase activity. The functional responses reported in both models were observed at time intervals which were considerably shorter than those used in the present study, 30 to 120 min. Support for this concept of *myo*-inositol metabolism was not found in the present study insofar as basal phosphatidylinositol levels were unaltered under hyperglycemic conditions. In the latter case, recent double labelling studies by Michell and co-workers have failed to support fully the concept of a hormone-sensitive pool in WRK-1 or other cell types in systems studied in the absence of insulin [32]. Comparable studies in the glomerular mesangial cell would have to be done to rule out this possibility. However, documenting the existence of hormone sensitive and insensitive pools is only important if functional changes exist between hyperglycemic and euglycemic conditions in the presence of normal or unchanged phosphoinositide levels. Because no detectable changes in intracellular calcium or InsP3 were observed under conditions of decreased bulk cellular inositol, there is no discrepancy between measured substrate (PIP2) and product (InsP3) and therefore no need to postulate the presence of such pools. There may exist tissues and cell lines in which *myo*-inositol is rate limiting for hormone-sensitive phosphoinositide synthesis and InsP3 formation under hyperglycemic conditions. These would provide a suitable model for assessing the potential importance of subcellular pools for *myo*-inositol turnover.

The data reported in the present study are consistent with the view that InsP3 formation is actually unrelated to bulk cellular *myo*-inositol levels and that, by implication, the changes in glomerular filtration may be unrelated to an inositol mediated signalling defect in InsP3 formation. This possibility is supported by the recent demonstration that *myo*-inositol levels are not statistically different in the glomeruli of diabetic rabbits under normoglycemic versus diabetic conditions [4].

In summary, direct chemical measurements of hormone stimulation in mesangial cells reveal no impair-

ment of InsP3 formation in cells grown under hyperglycemic conditions. Corresponding measurements of inositol glycerolipid content, cellular *myo*-inositol and phosphatidylinositol synthase fail to support a causal association between decreased cellular *myo*-inositol and impaired InsP3 formation.

Acknowledgments

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases grants DK 3955 and RO1 DK41487, and a Merit Review Award from the Veterans Administration.

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