J. Biochem. 122, 1196-1201 (1997)

# High Glucose-Induced Cytosolic Phospholipase $A_2$ Activation Responsible for Eicosanoid Production in Rat Mesangial Cells

**Misako Hayama, Satoshi Akiba, Misato Fukuzumi, and Takashi Sato[1]**

*Department of Pathological Biochemistry, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto, Kyoto 607*

Received for publication, July 28, 1997

**The stimulation of prostaglandin $E_2$ ($PGE_2$) production in mesangial cells exposed to a high glucose level was studied from the viewpoint of its implication in the glomerular hyperfiltration in diabetic nephropathy. The basal $PGE_2$ synthesis apparently increased in the cells on incubation with a high glucose level (20 mM) for 3-6 h. Under these conditions, secretory phospholipase $A_2$ activity was not detected in the incubation medium, but cytosolic phospholipase $A_2$ ($cPLA_2$) activity in the cells increased time-dependently up to 6 h, compared with that with a normal glucose level (5 mM). However, no difference in the $cPLA_2$ protein content between the two glucose levels was observed on immunoblot analysis, suggesting that the increased $cPLA_2$ activity under high glucose conditions is not due to stimulation of *de novo* synthesis. Stimulation with a calcium ionophore markedly enhanced arachidonic acid liberation and $PGE_2$ production by cells exposed to the high glucose level. Furthermore, mitogen-activated protein kinase (MAPK) activity increased time-dependently under high glucose conditions, the rate of increase being consistent with those in $cPLA_2$ activity and $PGE_2$ production under the same conditions. These data suggest that glucose-induced $cPLA_2$ activation through MAPK activation is responsible for the enhancement of $PGE_2$ production in mesangial cells.**

**Key words: cytosolic phospholipase $A_2$, high glucose level, mesangial cells, mitogen-activated protein kinase, prostaglandin $E_2$.**

Diabetic nephropathy, a major complication of diabetes mellitus, is characterized by the development of glomerular injury, such as hypertrophy and thickening of the basement membranes in the glomeruli. This pathogenesis has been proposed to be related to the dysfunction of glomerular mesangial cells under hyperglycemic conditions (*1, 2*).

Mesangial cells are responsible for eicosanoid production and are known to show increased synthesis of eicosanoids, which include pro-inflammatory lipid mediators such as prostaglandin $E_2$ ($PGE_2$), in response to elevated glucose levels in the cultured medium (*3*). The increased $PGE_2$ synthesis is associated with an increased glomerular filtration rate leading to urinary albumin excretion and, thus, is implicated in the pathogenesis of progressive glomerular dysfunction (*4, 5*).

A number of investigators have demonstrated that interleukin-1 or tumor necrosis factor-$\alpha$ stimulates $PGE_2$ synthesis, as well as arachidonic acid liberation, which results from an increase in mRNA for, and hence in the synthesis and secretion of, a secretory $PLA_2$ ($sPLA_2$) with a molecular mass of 14 kDa, in many types of cells (*6-8*) including glomerular mesangial cells (*9-17*). On the other hand, it has also been reported that rat mesangial cells have cytosolic $PLA_2$ ($cPLA_2$), a high molecular mass (85 kDa) $PLA_2$ having a preference for substrates containing arachidonic acid esterified at the *sn*-2 position of glycerophospholipids (*18-24*), and that $cPLA_2$ activation upon stimulation with cytokines, such as interleukin-1 or tumor necrosis factor-$\alpha$, is responsible for the enhanced $PGE_2$ synthesis (*21, 22*). Therefore, it is not completely clear which enzyme, $cPLA_2$ or $sPLA_2$, participates in the eicosanoid production in response to cytokines. However, recent studies indicate that in arachidonic acid liberation or PG synthesis, which occurs at a relatively early stage after stimulation, $cPLA_2$-dependent events are necessary for adequate activation of $sPLA_2$ in $P388D_1$ macrophages (*25*) or murine mast cells (*26*), suggesting the co-operative action of $cPLA_2$ and $sPLA_2$.

It was reported very recently that elevated glucose levels in the culture medium enhanced $cPLA_2$ activity in rat mesangial cells (*27*). Although the enhancement of $PGE_2$ synthesis is observed in rat mesangial cells cultured in high glucose medium, the contribution of $cPLA_2$ or $sPLA_2$ to the synthesis is also unclear.

With respect to the contribution of pro-inflammatory lipid mediators, such as $PGE_2$, to development of the pathogenesis of diabetic nephropathy, we investigated in the present work the effect of a high glucose level on the increased activity of $cPLA_2$ resulting in the enhanced arachidonic acid liberation and $PGE_2$ synthesis upon stimulation with the calcium ionophore, ionomycin, and the

[1] To whom correspondence should be addressed. Fax: +81-75-595-4759

Abbreviations: $cPLA_2$, cytosolic phospholipase $A_2$; MAPK, mitogen-activated protein kinase; $PGE_2$, prostaglandin $E_2$; PKC, protein kinase C; $sPLA_2$, secretory phospholipase $A_2$.

implication of mitogen-activated protein kinase (MAPK) in the increased enzyme activity.

## MATERIALS AND METHODS

*Materials*—RPMI 1640 was obtained from GIBCO BRL (Grand Island, NY, USA). The mixture of insulin, transferrin, and sodium selenite (ITS) was from Collaborative Biomedical Products (Bedford, MA, USA). BSA (fatty acid free) was from Nissui Pharmaceutical (Tokyo). Ionomycin was from Calbiochem (La Jolla, CA, USA). The $PGE_2$ enzyme immunoassay kit was from Cayman Chemical (Ann Arbor, MI, USA). Nitrocellulose membrane and peroxidase-conjugated goat anti rabbit antibody were from Bio-Rad Laboratories (Richmond, CA, USA). The MAPK assay kit and enhanced chemiluminescence (ECL) Western blotting detection kit were from Amersham (Buckinghamshire, UK). 1-Stearoyl-2-[$^3$H]arachidonoyl-glycerophosphocholine (160 Ci/mmol) and [$^3$H]arachidonic acid (100 Ci/mmol) were from New England Nuclear (Boston, MA, USA). All other chemicals were of reagent grade.

*Cell Culture*—Glomerular mesangial cells were prepared according to the method of Kikkawa *et al.* (*28*). Briefly, mesangial cells were obtained from a culture of glomeruli isolated from Sprague-Dawley rats (100–150 g) by sieving, and grown in RPMI 1640 supplemented with 20% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 $\mu$g/ml streptomycin, and ITS (5 $\mu$g/ml, 5 $\mu$g/ml, and 5 ng/ml, respectively). The cells (passage 3 to 10) were made quiescent by incubating them in RPMI 1640 containing 0.1 mg/ml BSA for 24 h. The quiescent cells were incubated in the test medium consisting of 0.1 mg/ml BSA and glucose-free RPMI 1640 supplemented with either 5 mM glucose, 20 mM glucose, or 5 mM glucose plus 15 mM L-glucose or raffinose.

*Assay for [$^3$H]Arachidonic Acid Release*—Confluent mesangial cells grown in 35-mm dishes were incubated with 0.5 $\mu$Ci/ml [$^3$H]arachidonic acid for 24 h in RPMI 1640 containing 0.1 mg/ml BSA. The labeled cells were washed with phosphate-buffered saline containing 0.1 mg/ml BSA, then incubated in the test medium. The cells were further incubated for 30 min in fresh test medium in the presence of 50 $\mu$M BW755C (3-amino-1-[*m*-trifluoromethyl)-phenyl]-2-pyrazolone, a cyclooxygenase and lipoxygenase inhibitor), then stimulated with 2 $\mu$M ionomycin for 15 min. The reaction was terminated by transferring the medium and the cell lysate, which was prepared by adding 0.1% SDS and 0.1 N NaOH, into ice-cold chloroform/methanol/HCl (200 : 200 : 1, v/v/v). Lipids in the medium and the lysate were extracted and separated by TLC on a Silica Gel G plate using diethyl ether/petroleum ether/acetic acid (80 : 80 : 2, v/v/v) as the developing system. Authentic arachidonic acid was co-chromatographed and each lipid fraction was visualized by exposing the plate to iodine vapor. The area corresponding to free fatty acid was scraped off and the radioactivity was determined. The amount of radioactive arachidonic acid released was expressed as a percentage of the total radioactivity.

*Assay for $PGE_2$ Production*—After exposure of mesangial cells to the test medium, the medium was removed for assay and replaced with fresh test medium. The cells were stimulated with 2 $\mu$M ionomycin for 15 min, then the medium was withdrawn. The quantity of $PGE_2$ in the medium was determined by using the enzyme immunoassay kit. The remaining cells were solubilized with 0.5 N NaOH and assayed for the protein content.

*Assay for $cPLA_2$ and MAP Kinase Activity*—Confluent mesangial cells grown in 100-mm dishes were incubated with the test medium for appropriate times. The cells were washed with an ice-cold buffer consisting of 100 mM NaCl, 1 mM $Na_3VO_4$, 20 mM $\beta$-glycerophosphate, 2 mM EGTA, 100 $\mu$M leupeptin, 100 $\mu$M *p*-(amidinophenyl)methanesulfonyl fluoride, and 10 mM Tris-HCl (pH 7.4), then scraped from the culture dish with a rubber policeman in the same buffer. After lysis of the cells by sonication, the lysate was centrifuged at 100,000 $\times g$ for 1 h at 4°C. The supernatant obtained was pretreated with 5 mM DTT at 37°C for 10 min to inactivate $sPLA_2$. The activity of $cPLA_2$ in the supernatant was determined by incubation with a mixture of 1-stearoyl-2-[$^3$H]arachidonoyl-glycerophosphocholine and unlabeled 1-stearoyl-2-arachidonoyl-glycerophosphocholine (0.5 Ci/mmol, 2 $\mu$M) as a substrate at 37°C for 1 h in the presence of 5 mM $CaCl_2$ and 100 mM Tris-HCl (pH 8.5). [$^3$H]Arachidonic acid released was extracted and determined as described by Sundaram *et al.* (*29*). The MAPK activity in the supernatant was measured using the MAPK assay kit.

*Preparation of Rabbit Antisera to Human $cPLA_2$ and Immunoblotting*—Rabbit antisera to human $cPLA_2$ were raised by intradermal injection of a peptide corresponding to amino acid residues 53–72 of human $cPLA_2$, generously donated by Dr. Kiso (Kyoto Pharmaceutical University). Mesangial cells incubated in the test medium for 6 h were subjected to SDS-PAGE (10% polyacrylamide gel) according to the method of Laemmli (*30*). The proteins were transferred to a nitrocellulose membrane, then the antiserum to $cPLA_2$ was applied. The bound antibodies were visualized using peroxidase-conjugated goat anti-rabbit antibodies and ECL reagent.

## RESULTS

*Basal $PGE_2$ Production under High Glucose Conditions*—Pro-inflammatory mediators including eicosanoids and cytokines are thought to be closely involved in the development of diabetic glomerular dysfunction. The accelerated eicosanoid production by mesangial cells, which plays a major role in the production in the glomeruli, causes functional abnormalities, such as glomerular hyperfiltration. Therefore, we examined the effect of a high glucose level on $PGE_2$ production by mesangial cells. When mesangial cells were exposed to normal (5 mM) or high (20 mM) glucose for 3 and 6 h, the $PGE_2$ produced increased remarkably after 3 h under high glucose conditions compared with under normal conditions (Fig. 1). The increase in $PGE_2$ production further increased after 6 h incubation under high glucose conditions.

*Glucose-Induced $cPLA_2$ Activation*—It is reasonable to consider that the increase in basal $PGE_2$ production by mesangial cells exposed to a high glucose level results from an increase in the activities of $PLA_2$ isozymes. In rat mesangial cells, two isozymes of $PLA_2$, $sPLA_2$ and $cPLA_2$, have been reported to exist (*24*). However, during incubation of the cells with normal or high glucose for 6 h, no detectable $PLA_2$ activity was observed in the extracellular medium of the cells (data not shown). This indicates that

the enhanced $PGE_2$ production under high glucose conditions is not due to the catalytic action of $sPLA_2$. Therefore, we investigated the $cPLA_2$ activity in cells exposed to a high glucose level. As shown in Fig. 2, DTT-insensitive $PLA_2$ activity increased time-dependently in the supernatant of the cell lysate obtained under high glucose conditions, indicating an increase in $cPLA_2$ activity in response to high glucose. Furthermore, immunoblot analysis with antiserum to $cPLA_2$ revealed almost no difference in the $cPLA_2$ proteins between cells exposed to normal and high glucose levels (Fig. 3), suggesting that the enhanced $cPLA_2$ activity under high glucose conditions is not due to an increase in *de novo* synthesis of the enzyme.

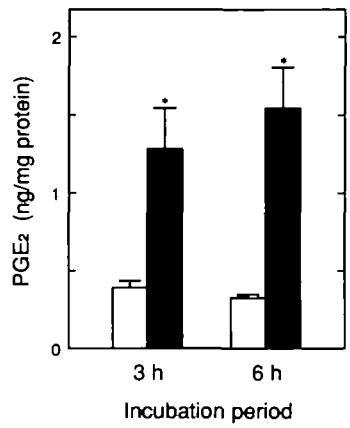


Fig. 1. **Effect of the glucose concentration on basal $PGE_2$ production in rat mesangial cells.** Confluent mesangial cells were incubated in test medium consisting of 0.1 mg/ml BSA and glucose-free RPMI 1640 supplemented with 5 mM glucose (open columns) or 20 mM glucose (closed columns) for the indicated periods. The medium was withdrawn and analyzed for $PGE_2$ production as described under "MATERIALS AND METHODS." Each bar represents the mean ± SE for quadruplicate determination. $^*p<0.02$, relative to corresponding response of the cells under 5 mM glucose.

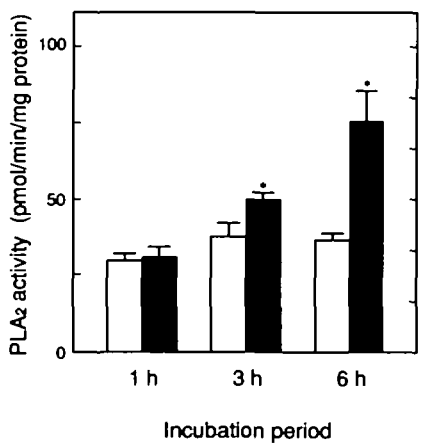


Fig. 2. **Effect of the glucose concentration on $cPLA_2$ activity in rat mesangial cells.** Confluent mesangial cells were incubated in the test medium (open columns, 5 mM glucose; closed columns, 20 mM glucose) for the indicated periods. The $cPLA_2$ activity in the supernatant of a cell lysate was determined as described under "MATERIALS AND METHODS." Each bar represents the mean ± SE for quadruplicate determination. $^*p<0.05$, relative to corresponding response of the cells under 5 mM glucose.

*$cPLA_2$-Catalyzed Arachidonic Acid Liberation and $PGE_2$ Production*—To confirm that the activated $cPLA_2$ could be responsible for the enhanced $PGE_2$ production seen in the mesangial cells under high glucose conditions, calcium ionophore ionomycin-induced arachidonic acid liberation and $PGE_2$ production were determined in cells exposed to high and normal glucose levels. As shown in Table I, arachidonic acid liberation and $PGE_2$ production under high glucose conditions were approximately twofold higher than those under normal glucose conditions, after 3 or 6 h of incubation with glucose. These increases in the time-dependent responses under high glucose conditions were in good agreement with the increase in $cPLA_2$ activity shown in Fig. 2.

It has been reported that osmotic stress could stimulate the MAPK cascade, which leads to $cPLA_2$ activation and $PGE_2$ production in MDCK cells (*31, 32*). The finding here that the exposure of mesangial cells to a high glucose level caused increases in arachidonic acid liberation and $PGE_2$ production is considered to be due to osmotic pressure caused by the high glucose concentration in the incubation medium. Therefore, we investigated the effects on these responses of L-glucose, which is also a poorly metabolized control, and raffinose by addition of 15 mM each to 5 mM glucose media. However, almost no increase in arachidonic acid liberation or $PGE_2$ production was seen under the osmotic pressure caused by these impermeable substances (Table I).

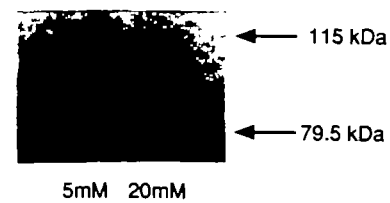


Fig. 3. **Effect of the glucose concentration on $cPLA_2$ protein in rat mesangial cells.** Confluent mesangial cells were incubated in the test medium (5 mM glucose or 20 mM glucose) for 6 h. The amount of $cPLA_2$ protein was determined by the immunoblot technique as described under "MATERIALS AND METHODS." The example shown is representative of two experiments.

TABLE I. **Effects of the glucose concentration on arachidonic acid liberation and $PGE_2$ production induced by ionomycin in rat mesangial cells.** [$^3$H]Arachidonic acid-labeled or unlabeled mesangial cells were incubated in the test media shown in the table for the indicated periods. Each medium was replaced with fresh medium, and the cells were stimulated with 2 $\mu$M ionomycin for 15 min. [$^3$H]-Arachidonic acid liberated and $PGE_2$ produced were determined as described under "MATERIALS AND METHODS." The data represent the means ± SE for quadruplicate determination. $^*p<0.01$, $^{**}p<0.02$, relative to corresponding response of the cells under 5 mM glucose. $^{***}$Not determined.

| Incubation medium | Arachidonic acid liberation (%) | | $PGE_2$ production (ng/mg protein) | |
|---|---|---|---|---|
| | 3 h | 6 h | 3 h | 6 h |
| 5 mM Glucose | 8.7±1.3 | 9.7±0.4 | 3.5±0.8 | 4.0±0.6 |
| 20 mM Glucose | 16.1±1.1* | 18.9±1.1* | 7.2±0.6** | 7.0±0.2* |
| 5 mM Glucose +15 mM L-Glucose | 9.0±1.0 | 12.3±0.9 | −*** | −*** |
| 5 mM Glucose +15 mM Raffinose | 8.1±0.7 | 11.2±1.2 | −*** | −*** |

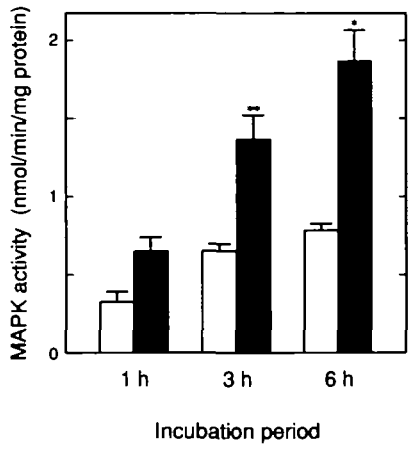


Fig. 4. **Effect of the glucose concentration on MAPK activity in rat mesangial cells.** The incubation conditions were as described in the legend to Fig. 2. The MAPK activity in the supernatant of a cell lysate was determined as described under "MATERIALS AND METHODS." Each bar represents the mean ± SE for quadruplicate determination. $^*p<0.01$, $^{**}p<0.05$, relative to corresponding response of the cells under 5 mM glucose.

*Glucose-Induced MAPK Activation*—Receptor-mediated $cPLA_2$ activation is well known to be regulated by the phosphorylation of the enzyme through MAPK activation (*33, 34*). Since it was reported that increased glucose levels stimulate MAPK activation, probably through PKC activation (*27*), MAPK activity was examined in mesangial cells under the high glucose conditions examined in the present work. As shown in Fig. 4, MAPK activity apparently increased time-dependently in the cells exposed to the high glucose level compared with that with a normal glucose level. The time-course of MAPK activation was well correlated with the $cPLA_2$ activation seen in Fig. 2, suggesting that high glucose-induced $cPLA_2$ activation is mediated through MAPK activation.

## DISCUSSION

Enhanced $PGE_2$ synthesis in renal glomuruli has been proposed to be associated with glomerular hyperfiltration characterized by an increased glomerular filtration rate, thus leading to the development of diabetic nephropathy, which is a major complication in patients with diabetes mellitus. Actually, it has been reported that isolated rat kidneys show increased prostaglandin synthesis with an increase in the glomerular filtration rate on perfusion with high glucose medium (*35*). Furthermore, with an *in vitro* system of cultured glomerular mesangial cells, a high glucose concentration was shown to induce increases in arachidonic acid liberation and $PGE_2$ generation within 3 h (*3*). Although it is not yet completely clear which isozyme of $PLA_2$s is involved in the mechanism, evidence was presented recently that high glucose conditions enhanced $cPLA_2$ activity *via* a PKC-dependent MAPK cascade in rat glomerular mesangial cells (*27*).

In the present work, we obtained evidence that when mesangial cells are exposed to a high glucose concentration, a significant increase in $PGE_2$ production occurs within the period of 3 to 6 h (Fig. 1). Under these conditions, the $cPLA_2$ activity in the supernatant from a lysate of cells exposed to a high glucose concentration apparently increased compared with that with a normal glucose level (Fig. 2). During incubation for 6 h, no $sPLA_2$ activity was detected in the supernatant of cells exposed to a high glucose concentration (data not shown). These data suggest, therefore, that $cPLA_2$ activation, but not $sPLA_2$ expression, is fully responsible for the high glucose-induced enhancement of $PGE_2$ production.

In an attempt to confirm that the elevated $PLA_2$ activity has the ability to hydrolyze membrane phospholipids, resulting in $PGE_2$ production, we obtained evidence that stimulation of cells exposed to a high glucose level with the calcium ionophore, ionomycin, led to approximately twice the arachidonic acid liberation and $PGE_2$ production observed for cells under normal glucose conditions (Table I). This finding strongly supports our suggestion mentioned above.

We confirmed further that the $cPLA_2$ proteins were the same in cells exposed to high and normal glucose levels, as revealed by immunoblotting with antiserum to $cPLA_2$, indicating that the increased $cPLA_2$ activity in cells exposed to elevated glucose level is not due to an increase in *de novo* synthesis of the enzyme. Therefore, the mechanism by which $cPLA_2$ activity increased under high glucose conditions is thought to involve enhanced signal transduction to $cPLA_2$ activation.

A number of studies have demonstrated that diabetes or high glucose levels induce increased *de novo* synthesis of diacylglycerol and PKC activity in glomeruli from normal or diabetic rats (*36, 37*), mesangial cells (*3, 27, 38, 39*), or other cultured cells (*40*). Inhibition of PKC activation with a specific inhibitor or the antisense has been reported to attenuate the increases in $PLA_2$ activity and $PGE_2$ production in mesangial cells in response to interleukin-1$\beta$ (*41*). These observations suggest that high glucose-induced PKC activation enhances the pathway upstream of $cPLA_2$ activation, that is, the MAPK cascade, thereby leading to increases in arachidonic acid liberation and $PGE_2$ production. In fact, it was reported recently that a MAPK kinase inhibitor suppressed glucose-induced $cPLA_2$ activation in mesangial cells (*27*). We also showed in the present work that cells exposed to a high glucose level showed marked enhancement of MAPK activity in parallel with the increase in $cPLA_2$ activity (Fig. 4). Here, we propose a mechanism for the MAPK activation leading to $cPLA_2$ activation in cells cultured under high glucose conditions.

Our data indicate that glucose-induced hyper-responses, *i.e.*, the activation of MAPK and $cPLA_2$, and the mobilization of arachidonic acid and $PGE_2$, apparently occur within 3-6 h and are, thus, relatively rapid. Glucose-induced PKC activation, as well as an increased diacylglycerol mass, was reported to be observed within 15 min in glomeruli (*37*) or 3 h in mesangial cells (*3*), although these responses were shown to occur after prolonged incubation (3-5 days) of mesangial cells with high glucose levels (*39*). Furthermore, it has been reported that interleukin-1$\alpha$ stimulated $cPLA_2$ activation within 10 min (*23*). Thus, the relatively rapid responses to high glucose levels or cytokines, *i.e.*, the production of pro-inflammatory lipid mediators, may reflect the initiation of diabetic nephropathy.

In the present work, we assessed the hydrolyzing activity of high glucose-activated $cPLA_2$ using a calcium ionophore. However, such an increase in intracellular $Ca^{2+}$ concentra-

tion is hardly considered to occur under high glucose conditions, because it is reported that stimulation of mesangial cells exposed to high glucose level with a receptor-mediated agonist, such as angiotensin II or vasopressin, did not induce a significant increase in intracellular $Ca^{2+}$ level and inositol phosphate production, which can mobilize intracellular $Ca^{2+}$, compared with those with a normal glucose level (*42, 43*). On the other hand, it is reported that interleukin-1 rapidly increased $cPLA_2$ activity through its phosphorylation but did not increase arachidonic acid release and $PGE_2$ formation, whereas interleukin-1 and transforming growth factor-$\beta_2$ in combination remarkably increased $PGE_2$ formation in mesangial cells (*21, 23*). A similar finding is reported with interleukin-1 and platelet-derived growth factor in mouse osteoblastic cells (*44*). On the basis of these findings, we consider that high glucose has a priming effect on $cPLA_2$ to enhance $PGE_2$ formation, like interleukin-1, and the functional responses of the primed $cPLA_2$ are characterized with calcium ionophore. The effect of calcium ionophore may reflect the responses with cytokines, such as transforming growth factor-$\beta_2$ or platelet-derived growth factor, which may present in inflammatory conditions under glomerular injury.

In conclusion, we have provided evidence that mesangial cells exposed to a high glucose level show the enhancement of MAPK and $cPLA_2$ activation, which results in increases in arachidonic acid liberation and $PGE_2$ production. We suggest that the increased production of pro-inflammatory lipid mediators is involved in the pathogenesis of glomerular hyperfiltration in diabetes mellitus under hyperglycemic conditions.

## REFERENCES


1. Mauer, S.M., Steffes, M.W., Ellis, E.N., Sutherland, D.E.R., Brown, D.M., and Goez, F.C. (1984) Structural-functional relationships in diabetic nephropathy. *J. Clin. Invest.* **74**, 1143-1155
2. Ziyadeh, F.N. and Goldfarb, S. (1991) The renal tubulointerstitium in diabetes mellitus. *Kidney Int.* **39**, 464-475
3. Williams, B. and Schrier, R.W. (1993) Glucose-induced protein kinase C activity regulates arachidonic acid release and eicosanoid production by cultured glomerular mesangial cells. *J. Clin. Invest.* **92**, 2889-2896
4. Craven, P.A., Caines, M.A., and DeRubertis, F.R. (1987) Sequential alterations in glomerular prostaglandin and thromboxane synthesis in diabetic rats: relationship to the hyperfiltration of early diabetes. *Metabolism* **36**, 95-103
5. Chang, W.P., Dimitriadis, E., Allen, T., Dunlop, M.E., Cooper, M., and Larkins, R.G. (1991) The effect of aldose reductase inhibitors on glomerular prostaglandin production and urinary albumin excretion in experimental diabetes mellitus. *Diabetologia* **34**, 225-231
6. Chang, J., Gilman, S.C., and Lewis, A.J. (1986) Interleukin 1 activates phospholipase $A_2$ in rabbit chondrocytes: a possible signal for IL 1 action. *J. Immunol.* **136**, 1283-1287
7. Kerr, J., Stevens, T.M., Davis, G.L., McLaughlin, J.A., and Harris, R.R. (1989) Effects of recombinant interleukin-1$\beta$ on phospholipase $A_2$ activity, phospholipase $A_2$ mRNA levels, and eicosanoid formation in rabbit chondrocytes. *Biochem. Biophys. Res. Commun.* **165**, 1079-1084
8. Oka, S. and Arita, H. (1991) Inflammatory factors stimulate expression of group II phospholipase $A_2$ in rat cultured astrocytes. Two distinct pathways of the gene expression. *J. Biol. Chem.* **266**, 9956-9960
9. Pfeilschifter, J., Pignat, W., Vosbeck, K., and Märki, F. (1989) Interleukin 1 and tumor necrosis factor synergistically stimulate prostaglandin synthesis and phospholipase $A_2$ release from rat renal mesangial cells. *Biochem. Biophys. Res. Commun.* **159**, 385-394
10. Schalkwijk, C., Pfeilschifter, J., Märki, F., and van den Bosch, H. (1991) Interleukin-1$\beta$, tumor necrosis factor and forskolin stimulate the synthesis and secretion of group II phospholipase $A_2$ in rat mesangial cells. *Biochem. Biophys. Res. Commun.* **174**, 268-275
11. Mühl, H., Geiger, T., Pignat, W., Märki, F., van den Bosch, H., Vosbeck, K., and Pfeilschifter, J. (1991) PDGF suppresses the activation of group II phospholipase $A_2$ gene expression by interleukin 1 and forskolin in mesangial cells. *FEBS Lett.* **291**, 249-252
12. Nakazato, Y., Simonson, M.S., Herman, W.H., Konieczkowski, M., and Sedor, J.R. (1991) Interleukin-1$\alpha$ stimulates prostaglandin biosynthesis in serum-activated mesangial cells by induction of a non-pancreatic (Type II) phospholipase $A_2$. *J. Biol. Chem.* **266**, 14119-14127
13. Pfeilschifter, J., Leighton, J., Pignat, W., Märki, F., and Vosbeck, K. (1991) Cyclic AMP mimics, but does not mediate, interleukin-1- and tumor-necrosis-factor-stimulated phospholipase $A_2$ secretion from rat renal mesangial cells. *Biochem. J.* **273**, 199-204
14. Schalkwijk, C., Pfeilschifter, J., Märki, F., and van den Bosch, H. (1992) Interleukin-1$\beta$- and forskolin-induced synthesis and secretion of group II phospholipase $A_2$ and prostaglandin $E_2$ in rat mesangial cells is prevented by transforming growth factor-$\beta$2. *J. Biol. Chem.* **267**, 8846-8851
15. Pfeilschifter, J., Schalkwijk, C., Briner, V.A., and van den Bosch, H. (1993) Cytokine-stimulated secretion of group II phospholipase $A_2$ by rat mesangial cells. Its contribution to arachidonic acid release and prostaglandin synthesis by cultured rat glomerular cells. *J. Clin. Invest.* **92**, 2516-2523
16. Walker, G., Kunz, D., Pignat, W., van den Bosch, H., and Pfeilschifter, J. (1995) Pyrrolidine dithiocarbamate differentially affects cytokine- and cAMP-induced expression of group II phospholipase $A_2$ in rat renal mesangial cells. *FEBS Lett.* **364**, 218-222
17. Vervoordeldonk, M.J.B.M., Schalkwijk, C.G., Pfeilschifter, J., and van den Bosch, H. (1996) Effects of dexamethasone and transforming growth factor $\beta$2 on group II phospholipase $A_2$ mRNA and activity levels in interleukin 1$\beta$- and forskolin-stimulated mesangial cells. *Biochem. J.* **315**, 435-441
18. Gronich, J.H., Bonventre, J.V., and Nemenoff, R.A. (1988) Identification and characterization of a hormonally regulated form of phospholipase $A_2$ in rat renal mesangial cells. *J. Biol. Chem.* **263**, 16645-16651
19. Bonventre, J.V., Gronich, J.H., and Nemenoff, R.A. (1990) Epidermal growth factor enhances glomerular mesangial cell soluble phospholipase $A_2$ activity. *J. Biol. Chem.* **265**, 4934-4938
20. Goppelt-Strübe, M. and Rehfeldt, W. (1992) Glucocorticoids inhibit TNF $\alpha$-induced cytosolic phospholipase $A_2$ activity. *Biochim. Biophys. Acta* **1127**, 163-167
21. Schalkwijk, C.G., de Vet, E., Pfeilschifter, J., and van den Bosch, H. (1992) Interleukin-1$\beta$ and transforming growth factor-$\beta_2$ enhance cytosolic high-molecular-mass phospholipase $A_2$ activity and induce prostaglandin $E_2$ formation in rat mesangial cells. *Eur. J. Biochem.* **210**, 169-176
22. Schalkwijk, C.G., Vervoordeldonk, M., Pfeilschifter, J., and van den Bosch, H. (1993) Interleukin-1$\beta$-induced cytosolic phospholipase $A_2$ activity and protein synthesis is blocked by dexamethasone in rat mesangial cells. *FEBS Lett.* **333**, 339-343
23. Gronich, J., Konieczkowski, M., Gelb, M.H., Nemenoff, R.A., and Sedor, J.R. (1994) Interleukin 1$\alpha$ causes rapid activation of cytosolic phospholipase $A_2$ by phosphorylation in rat mesangial cells. *J. Clin. Invest.* **93**, 1224-1233
24. Aarsman, A.J., Schalkwijk, C.G., Neys, F.W., Iijima, N., Wherrett, J.R., and van den Bosch, H. (1996) Purification and characterization of $Ca^{2+}$-dependent phospholipase $A_2$ from rat kidney. *Arch. Biochem. Biophys.* **331**, 95-103
25. Balsinde, J. and Dennis, E.A. (1996) Distinct roles in signal transduction for each of the phospholipase $A_2$ enzymes present in

P388D$_1$ macrophages. *J. Biol. Chem.* **271**, 6758-6765
26. Reddy, S.T. and Herschman, H.R. (1997) Prostaglandin synthase-1 and prostagalndin synthase-2 are coupled to distinct phospholipases for the generation of prostagalndin $D_2$ in activated mast cells. *J. Biol. Chem.* **272**, 3231-3237
27. Haneda, M., Araki, S., Togawa, M., Sugimoto, T., Isono, M., and Kikkawa, R. (1997) Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells cultured under high glucose conditions. *Diabetes* **46**, 847-853
28. Kikkawa, R., Umemura, K., Haneda, M., Arimura, T., Ebata, K., and Shigeta, Y. (1987) Evidence for existence of polyol pathway in cultured rat mesangial cells. *Diabetes* **36**, 240-243
29. Sundaram, G.S., Shakir, K.M.M., Barnes, G., and Margolis, S. (1978) Release of phospholipase A and triglyceride lipase from rat liver. *J. Biol. Chem.* **253**, 7703-7710
30. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685
31. Itoh, T., Yamauchi, A., Miyai, A., Yokoyama, K., Kamada, T., Ueda, N., and Fujiwara, Y. (1994) Mitogen-activated protein kinase and its activator are regulated by hypertonic stress in Madin-Darby canine kidney cells. *J. Clin. Invest.* **93**, 2387-2392
32. Terada, Y., Tomita, K., Homma, M.K., Nonoguchi, H., Yang, T., Yamada, T., Yuasa, Y., Krebs, E.G., Sasaki, S., and Marumo, F. (1994) Sequential activation of raf-1 kinase, mitogen-activated protein (MAP) kinase kinase, MAP kinase, and S6 kinase by hyperosmolality in renal cells. *J. Biol. Chem.* **269**, 31296-31301
33. Lin, L.-L., Wartmann, M., Lin, A.Y., Knopf, J.L., Seth, A., and Davis, R.J. (1993) cPLA$_2$ is phosphorylated and activated by MAP kinase. *Cell* **72**, 269-278
34. Nemenoff, R.A., Winitz, S., Qian, N.X., Van Putten, V., Johnson, G.L., and Heasley, L.E. (1993) Phosphorylation and activation of a high molecular weight form of phospholipase $A_2$ by p42 microtubule-associated protein 2 kinase and protein kinase C. *J. Biol. Chem.* **268**, 1960-1964
35. Craven, P.A., Patterson, M.C., and DeRubertis, F.R. (1988) Role of enhanced arachidonate availability through phospholipase $A_2$ pathway in mediation of increased prostaglandin synthesis by glomeruli from diabetic rats. *Diabetes* **37**, 429-435
36. Craven, P.A. and DeRubertis, F.R. (1989) Protein kinase C is activated in glomeruli from streptozotocin diabetic rats. Possible mediation by glucose. *J. Clin. Invest.* **83**, 1667-1675
37. Craven, P.A., Davidson, C.M., and DeRubertis, F.R. (1990) Increase in diacylglycerol mass in isolated glomeruli by glucose from *de novo* synthesis of glycerolipids. *Diabetes* **39**, 667-674
38. Ayo, S.H., Radnik, R., Garoni, J.A., Troyer, D.A., and Kreisberg, J.I. (1991) High glucose increases diacylglycerol mass and activates protein kinase C in mesangial cell cultures. *Am. J. Physiol.* **261**, F571-F577
39. Kikkawa, R., Haneda, M., Uzu, T., Koya, D., Sugimoto, T., and Shigeta, Y. (1994) Translocation of protein kinase C $\alpha$ and $\zeta$ in rat glomerular mesangial cells cultured under high glucose conditions. *Diabetologia* **37**, 838-841
40. Lee, T.-S., Saltsman, K.A., Ohashi, H., and King, G.L. (1989) Activation of protein kinase C by elevation of glucose concentration: Proposal for a mechanism in the development of diabetic vascular complications. *Proc. Natl. Acad. Sci. USA* **86**, 5141-5145
41. Rzymkiewicz, D.M., Tetsuka, T., Daphna-Iken, D., Srivastava, S., and Morrison, A.R. (1996) Interleukin-1$\beta$ activates protein kinase C$\zeta$ in renal mesangial cells. Potential role in prostaglandin $E_2$ up-regulation. *J. Biol. Chem.* **271**, 17241-17246
42. Menè, P., Pugliese, G., Pricci, F., Di Mario, U., Cinotti, G.A., and Pugliese, F. (1993) High glucose inhibits cytosolic calcium signaling in cultured rat mesangial cells. *Kidney Int.* **43**, 585-591
43. Hurst, R.D., Stevanovic, Z.S., Munk, S., Derylo, B., Zhou, X., Meer, J., Silverberg, M., and Whiteside, C.I. (1995) Glomerular mesangial cell altered contractility in high glucose is $Ca^{2+}$ independent. *Diabetes* **44**, 759-766
44. Chen, Q.-R., Miyaura, C., Higashi, S., Murakami, M., Kudo, I., Saito, S., Hiraide, T., Shibasaki, Y., and Suda, T. (1997) Activation of cytosolic phospholipase $A_2$ by platelet-derived growth factor is essential for cyclooxygenase-2-dependent prostaglandin $E_2$ synthesis in mouse osteoblasts cultured with interleukin-1. *J. Biol. Chem.* **272**, 5952-5958