PPAR\(\gamma\) ligands attenuate mesangial contractile dysfunction in high glucose

MAKI UETA, MASANORI WAKISAKA, TETSURO AGO, TAKANARI KITAZONO, UDAI NAKAMURA, MOTOTAKA YOSHINARI, MASANORI IWASE, and MITSUO IDA

Department of Medicine and Clinical Science, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan; and Internal Medicine, Kyushu Dental College, Kitakyushu, Japan

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Background. To elucidate the regulation of peroxisome proliferator-activated receptor \(\gamma\) (PPAR\(\gamma\)) and its roles in mesangial cells, we examined the expression of PPAR\(\gamma\)1 and effects of its ligands on cell phenotypes and angiotensin II–induced contractile response in cultured rat mesangial cells under a high (20 mmol/L) glucose condition.

Methods. The effects of tumor necrosis factor \(\alpha\) (TNF\(\alpha\)), protein kinase C (PKC) activation, antisense DNA for PPAR\(\gamma\)1, PPAR\(\gamma\)1 ligands and PD98059 were examined in mesangial cells cultured in either 5 mmol/L or 20 mmol/L glucose. The expressions of PPAR\(\gamma\)1 protein and \(\alpha\)-smooth muscle actin (\(\alpha\)SMA) as a marker of phenotype of cells were determined by Western blot. The expression of PPAR\(\gamma\)1 mRNA was determined by a reverse transcription-polymerase chain reaction method. The reduction of cell surface area in response to angiotensin II was measured by microscope to determine cellular contraction.

Results. PKC activation, TNF\(\alpha\), and 20 mmol/L glucose decreased PPAR\(\gamma\)1 at both protein and mRNA levels, which was inhibited by PD98059, a specific inhibitor of mitogen-activated protein kinase (MAPK). Decreases of PPAR\(\gamma\)1 protein and contractile response and an increase of \(\alpha\)SMA occurred simultaneously in the cells treated with 20 mmol/L glucose after 5 days, which were attenuated to the normal levels by PPAR\(\gamma\)1 ligands. The antisense DNA also induced the decrease of PPAR\(\gamma\)1 protein, contractile dysfunction, and increase of \(\alpha\)SMA.

Conclusion. MAPK suppresses PPAR\(\gamma\)1 at the transcriptional level, and the reduction of PPAR\(\gamma\)1 in cultured rat mesangial cells under the high glucose condition induces phenotypic change and loss of contractile function. PPAR\(\gamma\)1 ligands recover both reductions of PPAR\(\gamma\)1 protein and contractile response.

Peroxisome proliferator–activated receptor \(\gamma\) (PPAR\(\gamma\)) is a nuclear receptor and plays an important role in regulation of cell differentiation at transcriptional levels [1–3]. Fibroblasts are reported to differentiate to adipocytes by the activation of PPAR\(\gamma\) [1]. We also reported the expression of PPAR\(\gamma\)1 in rat mesangial cells and the function of PPAR\(\gamma\)1 to regulate the differentiation of the cells by showing changes of \(\alpha\)-smooth muscle actin expression [4]. Cell differentiation seems to regulate cell functions by changing the phenotypes of the cells. Phenotypic changes occur in many types of cells under various conditions [5, 6]. In vascular smooth muscle cells, two phenotypes, namely synthetic phenotype and contractile phenotype, are reported, and the former changed from the latter loses contractile function and induces cellular proliferation and protein production such as extracellular matrices [7, 8].

Glomerular hyperfiltration prior to pathologic changes such as glomerular basement membrane thickening and mesangial expansion seems to be the first change in diabetic nephropathy [9, 10]. With respect to the regulation of glomerular filtration, mesangial cells play an important role by altering their cellular tone [11, 12]. Mesangial cells during long-term high glucose conditions are reported to lose their contractile response to contracting substances such as angiotensin II and endothelin [13, 14]. Recent studies have shown that high glucose concentrations can induce phenotypic changes in mesangial cells [15, 16]. The phenotypic change in mesangial cells is considered to occur in the early stage of diabetes in vivo [16].

Activation of PPAR\(\gamma\) by its ligands has been reported to induce beneficial effects on PPAR\(\gamma\) expressing cells by modulating their phenotype. PPAR\(\gamma\) ligands inhibit macrophages in atherosclerosis and inflammatory functions of activated monocytes in rheumatoid arthritis, and induce apoptosis in neoplasms such as lung and breast cancer [17–20]. Thiazolidinediones and 15deoxy-12, 14 delta-prostaglandin J2 (15dPGJ2) are synthesized PPAR\(\gamma\) ligands and natural PPAR\(\gamma\) ligand, respectively [21]. Thiazolidinediones have been used as antidiabetic drugs, and were reported to result in amelioration of diabetic nephropathy without affecting blood glucose levels.

Key words: PPAR\(\gamma\), phenotype, diabetic nephropathy, mesangial cell, contraction, thiazolidinediones.

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and blood pressure in streptozotocin-induced diabetic rats [22]. Recently, some of prostaglandin-synthesis inhibitors such as indomethacin and fenoprofen are reported to act as PPARγ ligands, while acetyl salicylic acid does not [23].

Based on these observations, it is possible that PPARγ1 in mesangial cells may be involved in the contractile dysfunction of the cells under high glucose environment by changing the cellular phenotype through activation of PPARγ1. However, whether the expression of PPARγ1 actually changes phenotype of the cells under high glucose environment has not yet been determined.

The aim of the present study was done to determine the expression PPARγ1 in the presence of high glucose concentrations, and to investigate whether activation of PPARγ1 by its ligands attenuates the reduced contractile response of rat mesangial cells under the high glucose condition.

METHODS

Cell preparation

Mesangial cells were isolated from kidneys of four-week-old Sprague-Dawley rats by the differential sieving procedure reported previously [24]. The cells were cultured in plastic plates (Nunc Brand Products, Roskilde, Denmark) in a 1:1 mixture of Dulbecco’s modified Eagle’s medium (DMEM) and Ham’s F-12 (both from Sigma, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY, USA), 10% Nu-serum (Collaborative Research, Bedford, MA, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL) (Sigma). Rat mesangial cells at passages 3–5 from five different preparations were used for the following experiments.

Western blots of PPARγ1 and α-smooth muscle actin

Rat mesangial cells on 60 mm plastic plates (Nunc) were cultured with DMEM-F12 containing 10% FBS and 10% Nu-serum with either 5 or 20 mmol/L glucose in the presence or absence of 10−6 mol/L pioglitazone, 10−5 mol/L 15dPGJ2, a natural PPARγ ligands, 2 × 10−5 mol/L indomethacin 10−4 mol/L acetyl salicylic acid and 4 × 10−6 mol/L PD98059, which inhibits the activation of mitogen-activated protein kinase (MAPK) by mitogen-activated protein kinase kinase (MAPKK) [25], for 5 days. The effect of protein kinase C (PKC) activation with preincubation of 10−8 mol/L of 12-O-tetradecarbonyl 13-acetate (TPA) for 20 minutes and the effect of 10−11 mol/L tumor necrosis factor α (TNFα) on PPARγ1 protein were also examined in the presence and absence of PD98059 in DMEM-F12 with 5 mmol/L glucose. The medium was changed every day. Western blotting of PPARγ1 and α-smooth muscle actin was performed as described previously [4]. Briefly, mesangial cells were harvested and lysed in a solution containing 20 mmol/L Tris/HCl, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L DTT, 1 mmol/L phenylmethylsulfonfluoride (PMSF), 1% Nonidet P-40 and 1% Triton X. Protein extracts were obtained by centrifugation of the lysate. The protein extract (25 μg) and positive controls of PPARγ1 protein from Jurkat whole cell lysate (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were separated on 10% non-gradient acryl amide gel using sodium dodecyl acryl amide gel electrophoresis, then transferred to nitrocellulose membranes. Rabbit immunoglobulin G (IgG) antibodies against PPARγ1 and α-smooth muscle actin (both Santa Cruz Biotechnology) were reacted to nitrocellulose membranes, followed by peroxidase-linked protein A (Amersham Life Science, Buckinghamshire, UK). Immunoreactive bands were visualized by Western blotting detection electrochemiluminescence (ECL) reagents (Amersham) with the use of Xomatic AR film (Eastman-Kodak, Rochester, NY, USA).

Cellular contraction experiment

Cellular contraction experiment was carried out by the method previously described [26]. Briefly, rat mesangial cells were incubated in DMEM-F12 with 10% FBS and 10% Nu-serum containing either 5 or 20 mmol/L glucose in the presence and absence of appropriate concentrations of pioglitazone, troglitazone, indomethacin, acetyl salicylic acid, and 15dPGJ2 and PD98059 up to 5 days. The medium was changed every day. Effects of angiotensin II on the tonus of cells were examined by measuring the surface area of the cells. The cellular surface area was measured using cells cultured on 6-well plates. The cells were preincubated in DMEM-F12 with 5 mmol/L glucose containing 10% FBS and 10% Nu-serum for 1 hour at 37°C in 95% air 5% CO₂ gas mixture. Cells cultured on 6-well plates were incubated in 20 mmol/L Tris/HEPES (pH 7.4) buffer containing (in mmol/L) glucose 5, NaCl 145, KCl 5, MgSO₄ 2.5, and CaCl₂ 1 in the presence of 10−7 mol/L angiotensin II after washing three times with the same buffer. The cells were recorded by a light-microscope equipped with a digital camera (Nikon, Tokyo, Japan) for 30 minutes. Serial changes in the surface area were calculated using NIH image software (written by Wayne Rasband).

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was prepared from rat mesangial cells cultured in the conditioned media using Isogen (Wako Junyaku, Osaka, Japan). To amplify the PPARγ1 and GAPDH cDNA fragments, PCR primers for PPARγ1 and GAPDH were synthesized as follows: forward primer sequences (5′-CCTCTCTCTGATGGATGACC-3′) and (5′-TGAACGGGAAGCT CACTGG-3′), and reverse
RT-PCR products of PPAR
corem were mixed with a loading buffer and then were elec-
tively. The cells were washed 3 times with DMEM-
F12, and then were incubated in DMEM-F12 containing
5 mmol/L glucose and 20% FBS for 48 hours. The cells on
60-mm plates were harvested to examine α-smooth mus-
cle actin and PPARγ1. The cells on 6-well plates were
used for the cellular contraction study.

**Other determinations**

To determine cell viability, confluent mesangial cells
on 96-well plate cultured in the media with different con-
centrations of pioglitazone for 5 days were used. The
viability was quantified by 3-(4,5-dimethylthiazol-2-yl)-
2,5-difenyltetrazolium bromide (MTT) assay purchased
from Promega. Densitometric analyses were conducted
using NIH image program.

**Statistical analysis**

Data were expressed as mean ± SEM. Differences be-
tween groups were examined for statistical significance
using analysis of variance (ANOVA) and paired and un-
paired Welch’s t test (two-tailed). A P value less than
0.01 denoted the presence of a statistically significant
difference.

**RESULTS**

Expression of PPARγ1 and of α-smooth muscle actin

The effect of TNFα and activation of PKC on PPARγ1
protein is shown in Figure 1. TNFα suppressed the
PPARγ1 protein after 24 hours. The PPARγ1 protein
was not change 1 day after activation of PKC (data not
shown); however, the protein decreased significantly af-
fer 2 days. The decreased PPARγ1 protein by both TNFα

![Graph showing relative density of PPAR γ1 protein](image)

**Antisense DNA for PPARγ1**

Antisense DNA and sense DNA for PPARγ1 were
synthesized as follows: antisense DNA sequences
(5′-TTTCTACTCTTTTGTGAT-3′) and (5′-TACAGCAACAGGGTGTTGA-3′),
respectively. RT-PCR was performed using 1 µg of total
RNA from rat mesangial cells under different condi-
tions, an Oligo (dT) 15 primer (Promega, Madison, WI,
USA) and 0.5 µmol/L of each primer, using Ready-To-Go
RT-PCR Beads (Amerham Pharmacia Biotech, Inc.,
Piscataway, NJ, USA). Reverse-transcription to cDNA
was performed at 42°C for 30 minutes. The temperature
program for the PCR amplification was 25 cycles of 30 sec-
onds at 94°C, 30 seconds at 54°C, and 30 seconds at 72°C
for PPARγ1, and 19 cycles of 30 seconds at 94°C, 30 sec-
onds at 58°C, and 30 seconds at 72°C for GAPDH, using
a thermal cycler (model 9600, Perkin Elmer-Cetus, Foster
City, CA, USA). Each 8 µL of both PCR products
were mixed with a loading buffer and then were elec-
trophoresed in a 2% agarose gel. To confirm linearity of
RT-PCR products of PPARγ1 and GAPDH, 0.5, 1, 2, 3 µg
of the stocked total RNA from mesangial cells cultured
in 5 mmol/L glucose was used.

![Graph showing relative density of PPAR γ1 protein](image)
and PKC activation was attenuated by the inhibition of MAPK with PD98059. The decreased protein by PKC activation was also attenuated by pioglitazone. The serial changes in PPARγ1 expressions of mesangial cells in 5 or 20 mmol/L glucose are shown in Figure 2. The expression of PPARγ1 protein in cells treated with 20 mmol/L glucose did not change until 3 days, and decreased significantly after 5 days compared with that in 5 mmol/L glucose. The effects of PPARγ1 ligands and PD98059 on PPARγ1 protein of cultured cells for 5 days are shown in Figure 3. Pioglitazone significantly increased PPARγ1 protein compared to that in 5 mmol/L glucose. Pioglitazone, 15dPGJ2 and indomethacin prevented the suppression of PPARγ1 protein expression in 20 mmol/L glucose. However, acetyl salicylic acid had no effect on PPARγ1 protein. MAPK inhibition by PD98059 also normalized the expression of PPARγ1 in 20 mmol/L glucose. Effects of high glucose, TNFα, and PKC activation on PPARγ1 mRNA are shown Figure 4. These factors significantly suppressed the mRNA, and the suppressed mRNA expressions were attenuated by the addition of PD98059. Pioglitazone increased PPARγ1 mRNA in 5 mmol/L and 20 mmol/L glucose. Treatment of the cells with 20 mmol/L glucose increased α-smooth muscle actin, and this increased α-smooth muscle actin was normalized by pioglitazone and indomethacin, but not by acetyl salicylic acid (Fig. 5).

**Effect of angiotensin II and high glucose on cellular contraction**

Representative photomicrographs of changes in surface area of mesangial cells in response to angiotensin II are shown in Figure 6. Angiotensin II decreased surface area of mesangial cells in a dose-dependent manner. Angiotensin II more than 10⁻⁸ mol/L significantly reduced the area (Fig. 7A). The contractile response of the cells in 20 mmol/L glucose to angiotensin II did not differ from that in 5 mmol/L glucose until 3 days of incubation, and then decreased significantly after 5 days compared to those in 5 mmol/L glucose and in 20 mmol/L glucose after 1 and 3 days incubation (Fig. 7B).

**Effect of pioglitazone on angiotensin II-induced contraction of rat mesangial cells in 5 and 20 mmol/L glucose**

Figure 8A demonstrates the effect of various concentrations of pioglitazone on changes in surface area of mesangial cells cultured in 5 or 20 mmol/L glucose after 5 days. The contractile response of the cells to angiotensin II under both glucose conditions was significantly reduced in the presence of higher concentrations of pioglitazone (10⁻⁴ and 10⁻⁵ mol/L). However, 10⁻⁶ and 10⁻⁷ mol/L of pioglitazone prevented the reduction of the contractile response in 20 mmol/L glucose. Effect of pioglitazone on viability of rat mesangial cells by MTT assay is shown in Figure 8B. The viability of the cells in 5 mmol/L and 20 mmol/L glucose did not change at the lower concentrations of pioglitazone up to 10⁻⁵ mol/L; however, 10⁻⁴ mol/L of pioglitazone significantly decreased these viability of the cells, compared to that in 5 mmol/L glucose without pioglitazone.

**The effects of 15dPGJ2, indomethacin, PD98059, and acetyl salicylic acid on the reduced contractile response**

15dPGJ2, indomethacin, and PD98059 normalized the reduced contractile response in 20 mmol/L glucose to the response in 5 mmol/L glucose, respectively. However, acetyl salicylic acid (10⁻⁴ mol/L) had no effect on the reduced contractile response in 20 mmol/L glucose (Fig. 9).

**Effect of antisense DNA for PPARγ1 on PPARγ1 protein, α-smooth muscle actin, and contractile response**

Both 0.5 and 1.0 μmol/L of antisense DNA significantly suppressed PPARγ1 protein and increased α-smooth muscle actin, compared with those in DMEM-F12 with 0.5 and 1.0 μmol/L of sense DNA (Fig. 10A and B). Angiotensin II induced cellular contraction, and a significant 35% decrease of surface area in 5 mmol/L glucose alone was observed (Fig. 10C). Antisense DNA
suppressed completely the contractile function of mesangial cells. In the presence of sense DNA of 0.5 μmol/L and 1.0 μmol/L, angiotensin II significantly decreased the surface area and the degrees of the decrease were 29% and 23%, respectively. The degree of the decrease of surface area in 1.0 μmol/L sense DNA was significantly less compared with that in 5 mmol/L glucose alone.

**DISCUSSION**

In the regulation of PPARγ, tumor necrosis factor-α (TNF-α) has been reported as an important factor to suppress PPARγ at both protein and mRNA level in preadipocytes [27–30]. Low levels of PPARγ1 mRNA and protein have been reported in human macrophage cultured in high glucose [31]. We demonstrated that high...
glucose as well as TNF-α and PKC activation decreased PPARγl at both the protein and mRNA levels in rat mesangial cells. The suppression of PPARγl by these factors was normalized by the inhibition of MAPK. These results indicate MAPK activation down-regulates PPARγl expression at the transcriptional level. The activation of MAPK by TNFα and the suppression of PPARγ action by MAPK have been reported in macrophage [32, 33]. In high glucose environments, activation of the DAG-PKC-ERK pathway has been reported to occur in rat mesangial cells [34]. The mechanism of the suppression of PPARγl under the high glucose condition seems to be explained by the activation of DAG-PKC-ERK pathway. The high glucose-induced PKC activation has been reported to occur after 3 days in rat aortic smooth muscle cells [35], which may explain the delay in suppressing PPARγl protein under the high glucose condition.

We also demonstrated pioglitazone induced PPARγl at both protein and mRNA levels. The induction of PPARγl at mRNA and protein levels by troglitazone [36] and the prevention of TNF-α–induced suppression in PPARγl mRNA by troglitazone, pioglitazone, and 15d-PGJ2 [28, 37] have been also reported. We reported that troglitazone normalized the increased cellular redox potential in rat mesangial cells in high glucose [38]. This effect of troglitazone may normalize PKC activity, since the increased cellular redox potential is reported to activate PKC activity [39]. Pioglitazone has been reported to normalize PKC activity in rat kidney [22]. The normalization of PKC activity may normalize MAPK activity in the DAG-PKC-ERK pathway. Indomethacin has been reported to inhibit MAPK [40]; however, 15dPGJ2 and thiazolidinediones have been reported to activate MAPK recently [41, 42]. These reports indicate PPARγ ligands other than indomethacin directly promote PPARγ protein at the level of gene expression independent of MAPK activity.

We examined α-smooth muscle actin as a marker of phenotypes of mesangial cells. α-Smooth muscle actin is not present in normal glomeruli and emerges in diseased glomeruli such as diabetic nephropathy [43]. PPARγ ligands reversed the increased α-smooth muscle actin in 20 mmol/L glucose to the level in 5 mmol/L glucose. These results suggest that PPARγ in rat mesangial cells also regulates phenotypes of the cells as reported in adipocytes [1, 29, 44], and the phenotypic change by the suppression of PPARγl under the high glucose condition is recovered to the phenotype under the normal glucose condition by PPARγ ligands.

We also demonstrated the contraction of mesangial cells by measuring changes in the surface area of the cells, as reported previously [26]. The response of the cells to angiotensin II was similar to those described in previous studies [45]. Interestingly, the contractile response of the cells in 20 mmol/L glucose to angiotensin II decreased after 5 days incubation, which coincided with the decrease in PPARγl protein. Moreover, the decreased contractile response was attenuated by the normalization of PPARγl protein with PPARγ ligands. These results indicate that the suppression of PPARγ1 protein under the high glucose condition induces the contractile dysfunction of mesangial cells by changing the phenotype of the
cells, since phenotypic change is important for cells to exhibit their functional properties, such as cellular contraction [7, 8, 46].

A recent study raised PKC-mediated cytoskeletal dysfunction as a mechanism for the decreased contractile response in high glucose [47]. In this study, the decreased contractile response in high glucose was also recovered by the inhibition of MAPK with PD98059. Because MAPK positioned the downstream of PKC, activation of MAPK may be important for the decreased contractile response. However, PPARγ ligands have been reported to activate MAPK as mentioned above [41, 42]. The suppression of PPARγ1 protein by the activation of MAPK rather than the MAPK activation itself seems to be the cause of the high glucose–induced contractile dysfunction of the cells. Moreover, we demonstrated the suppression of PPARγ1 protein by the antisense DNA for PPARγ1, that we made, induced an increase of α-smooth muscle actin and contractile dysfunction of mesangial cells in 5 mmol/L glucose. These results indicate that PPARγ1 acts as a regulator of phenotype of mesangial cells and that the decrease of PPARγ1 action by the suppression of its protein level induces de-differentiation of the cells, of which contractile function is decreased simultaneously.

The concentration of pioglitazone, which attenuates the high glucose–induced contractile dysfunction, is similar to the serum concentrations in diabetic patients treated with the same drug. Isshiki et al reported that pioglitazone recovered the glomerular hyper filtration, which is observed in the early stage of diabetic nephropathy, without affecting the sustained high blood glucose level in diabetic rats [22]. Normalization of PPARγ1 protein by its ligands under the high glucose condition may contribute the beneficial effect on glomerular filtration by keeping the cellular phenotypes with normal contractile function.

We demonstrated the higher concentrations of pioglitazone more than $10^{-5}$ mol/L decreased the contractility of rat mesangial cells in this study. Similar to pioglitazone, the high concentration of troglitazone ($2.5 \times 10^{-6}$ mol/L) decreased the contractility of the cells, and lower concentration of the drug ($2.5 \times 10^{-7}$ mol/L) prevented the reduced contractile response to in 20 mmol/L glucose (data not shown). The cell viability did not change at the concentration of pioglitazone up to $10^{-5}$ mol/L; however, it significantly decreased at $10^{-4}$ mol/L of the drug. Guan et al reported the similar effect of troglitazone on mesangial cells, and $2 \times 10^{-5}$ mol/L of troglitazone decreased the cell viability, while $1 \times 10^{-5}$ mol/L of troglitazone did not [48]. Pioglitazone and troglitazone have been reported to act as calcium channel blockers in smooth muscle cells, and those concentration were reported $3.0 \times 10^{-6}$ mol/L and $4.5 \times 10^{-5}$ mol/L, respectively [49, 50]. Recently, pioglitazone and troglitazone more than $10^{-5}$ mol/L were also reported to suppress angiotensin II receptor at the transcriptional level [51]. These effects of pioglitazone on cell viability, calcium channel, and angiotensin II receptor may explain the suppression of the angiotensin II-induced cellular contraction at the higher concentration of the drug.
Fig. 7. Changes in surface area of rat mesangial cells in the presence of different concentrations of angiotensin II (A) and effect of high glucose on $10^{-7}$ mol/L angiotensin II-induced contraction of cells during incubation in 20 mmol/L glucose for 5 days (B). Data are mean ± SEM of 20 to 30 cells from 3 separate experiments. $^*P < 0.01$ vs. 5 mmol/L glucose (no agonist) $^\dagger P < 0.01$ vs. 5 mmol/L glucose + angiotensin II. $^\ddagger P < 0.01$ vs. 20 mmol/L glucose + angiotensin II.

Fig. 8. Effect of pioglitazone on angiotensin II-induced contraction (A) and cell viability (B) of cells cultured in either 5 mmol/L or 20 mmol/L glucose for 5 days. The changes in cell surface area in response to $10^{-7}$ mol/L angiotensin II were analyzed. Data are mean ± SEM of 20 to 30 cells from 3 separate experiments. $^*P < 0.01$ vs. 5 mmol/L glucose + angiotensin II. $^\ddagger P < 0.01$ vs. 20 mmol/L glucose + angiotensin II. (B) The cell viability was analyzed using MTT assay as described in Methods. $N = 6$ of each condition. Data are mean ± SEM $^*P < 0.01$ vs. 5 mmol/L glucose. $^\dagger P < 0.01$ vs. 20 mmol/L glucose.

Fig. 9. Effects of $10^{-5}$ mol/L 15 deoxy-12, 14 delta-prostaglandin J2 (15dPDJ2), $2 \times 10^{-5}$ mol/L indomethacin (indo), $10^{-4}$ mol/L acetyl salicylic acid (Asp), and $4 \times 10^{-6}$ mol/L PD98059 on angiotensin II-induced contraction and change in surface area of rat mesangial cells after 5 days incubation in 20 mmol/L glucose. Data are mean ± SEM of 20 to 30 cells from 3 separate experiments. $^*P < 0.01$ vs. 5 mmol/L glucose + angiotensin II. $^\ddagger P < 0.01$ vs. 20 mmol/L glucose + angiotensin II after 5 days incubation.
The role of prostaglandin E2 on the reduced contractile response of mesangial cells in high glucose has been reported, since prostaglandin E2 decreases mesangial cell tonicity, and an increased production of prostaglandin E2 by mesangial cells in high glucose has also been reported [52, 53]. The concentrations of indomethacin and acetyl salicylic acid used in our study were sufficiently high to suppress prostaglandin synthesis [54, 55]; however, only indomethacin as a PPARγ ligand could attenuate the reduced contractile response. These data suggest that the reduced contractile response of cultured mesangial cells in high glucose is independent of prostaglandin E2.

CONCLUSION

We demonstrate that PPARγ ligands attenuated the decreased contractile response of mesangial cells under the high glucose condition with the recovery of PPARγ protein and phenotypes of the cells.

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Reprint requests to Masanori Wakisaka, M.D., Department of Medicine and Clinical Science, Graduate School of Medical Science, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka, 812–8582, Japan.
E-mail: mwaki@kyu-dent.ac.jp
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