Transforming Growth Factor-β1 Inhibits Type I Inositol 1,4,5-Trisphosphate Receptor Expression and Enhances Its Phosphorylation in Mesangial Cells*

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A potentially important cross-talk characteristic of transforming growth factor-β (TGF-β) is to inhibit platelet-derived growth factor-induced intracellular calcium rise (Baffy, G., Sharma, K., Shi, W., Ziyadeh, F. N., and Williamson, J. R. (1995) Biochem. Biophys. Res. Commun. 210, 378–383) in murine mesangial cells. The present study examined the possible basis for this effect by evaluating the regulation of the type I inositol 1,4,5-trisphosphate receptor (IP₃R) by TGF-β. TGF-β1 down-regulates IP₃R protein expression by >90% with maximal and half-maximal effects after 8 and 2 h, respectively. TGF-β1 also decreased IP₃R mRNA expression by 59% after 1 h. Phosphorylation of the IP₃R was also demonstrated as early as 15 min after TGF-β1 exposure. Back phosphorylation assays of IP₃R from TGF-β1-treated mesangial cells with protein kinase A (PKA), indicated that TGF-β1-induced phosphorylation of the IP₃R occurs at similar sites as for PKA. In vitro kinase assays using the known IP₃R peptide substrates for PKA, RPS-GRESlTSGFNP and ARRDSVLAA, demonstrated that TGF-β1 induces phosphorylation of both peptides (158 and 123% of control values, respectively). TGF-β1-induced phosphorylation was prevented by the addition of the PKA inhibitor peptide in the in vitro kinase assay. It is proposed that TGF-β1-mediated effects on the IP₃R may be an important characteristic of its ability to modulate the response of cells to factors that employ IP₃R-mediated calcium release.

Transforming growth factor (TGF)³-β1 has been implicated in a variety of inflammatory and noninflammatory kidney diseases (1). Our prior studies have demonstrated that TGF-β1 is up-regulated in animal models of diabetic kidney disease (2) and inhibition of TGF-β activity by neutralizing antibodies reduces diabetic renal hypertrophy and gene expression of type IV collagen and fibronectin (3). Apart from its well described effects to stimulate matrix production and regulate cell growth, TGF-β also has a characteristic effect to modulate the phenotypic actions of other factors. In particular, TGF-β can inhibit the proliferative ability of PDGF and other mitogens in human mesangial cells (4). This is of particular relevance to many glomerular diseases in that up-regulation of multiple growth factors is observed concomitantly (5, 6).

Studies in a variety of cell types have demonstrated a modulatory capacity of TGF-β to affect the cellular response to exogenous factors. TGF-β inhibits PDGF-induced proliferation and inositol 1,4,5-trisphosphate (IP₃) production in human bone marrow fibroblasts (7). In studies with cardiac fibroblasts (8) and vascular smooth muscle cells (9), pretreatment with TGF-β1 for at least 30 min inhibits [Ca²⁺]ᵢ release in response to isoproterenol and angiotensin II, respectively. In our studies with transformed murine mesangial cells, we demonstrated that TGF-β markedly inhibits PDGF-BB-induced increase in [Ca²⁺]ᵢ, (10).

A common pathway of raising [Ca²⁺]ᵢ, with PDGF, isoproterenol, and angiotensin II is the activation of phospholipase Cγ or phospholipase Cβ and the generation of IP₃ from phosphatidylinositol 4,5-bisphosphate (11). The raised IP₃ levels bind to IP₃ receptors (IP₃R) in the endoplasmic reticulum to release stored calcium into the cytoplasmic space (11). This is thought to be a crucial step in allowing the cell to respond to these agonists. There are at least three isoforms of the IP₃ receptor, derived from three distinct genes (reviewed in Ref. 12). The type I isoform is abundant in cerebellum but is also present in many peripheral tissues. Alternative splicing of the type I IP₃R results in deletion of the SII segment in nonneural tissues (13, 14). The SII segment is present between two serines (serine 1589 and serine 1755) (13, 14), which are phosphorylated by protein kinase A (PKA) (14, 15). Phosphorylation of the cerebellar type I IP₃R by PKA impairs [Ca²⁺]ᵢ, mobilization by IP₃ (16, 17). In addition, muscarinic receptor activation impairs IP₃-induced [Ca²⁺]ᵢ, mobilization by enhancing the degradation of the type I isoform in neuroblastoma cells (18, 19). The type III isoform of the IP₃R has been recently found to be up-regulated during apoptosis of T-lymphocytes (20). The type I isoform of the IP₃R is present in the glomerulus of the kidney (21), primarily in mesangial cells (22), as well as in the renal vascular system (23). Other isoforms of the IP₃R have not been identified in the glomerulus (21). Since TGF-β appears to modulate IP₃-induced [Ca²⁺]ᵢ, mobilization in a variety of cell types, we postulate that TGF-β may mediate some of its effects via regulation of the IP₃R.

Our present study demonstrates that TGF-β inhibits the protein expression of the type I isoform of the IP₃R in mesangial cells and that this may be partly due to a decrease of the

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¶ The abbreviations used are: TGF, transforming growth factor; IP₃, inositol 1,4,5-trisphosphate; IP₃R, IP₃ receptor; PDGF, platelet-derived growth factor; MMC, murine mesangial cells; PBS, phosphate-buffered saline; DEEM, Dulbecco’s modified Eagle’s medium; PKA, protein kinase A; PKC, protein kinase C; PMSF, phenylmethylsulfonyl fluoride; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; bp, base pair.
steady-state mRNA level. In addition, TGF-β rapidly phosphorylates the IP3R. The likely consequence of these effects is to modulate the IP3R function and thus affect the cellular responsiveness to agents that act via activation of the IP3R.

**EXPERIMENTAL PROCEDURES**

**Materials—**[32P]Inorganic phosphate, [γ-32P]ATP, and [γ-32P]CTP were from DuPont NEN. An enhanced chemiluminescence kit was purchased from Amersham Corp. TGF-β1 was purchased from R & D Systems. All other reagents were from Sigma unless otherwise noted.

**Culture—**An SV40-transformed murine glomerular mesangial cell line (MMC) that has been previously described (24) was primarily used in these studies. These cells retain many of the differentiated characteristics of mesangial cells in primary culture (24). To assure that our findings were not influenced by transformation, we performed a limited series of experiments in rat glomerular mesangial cells that were conducted between passages 4 and 10. Isolation of rat mesangial cells was performed as detailed previously (25).

**Time Course of TGF-β1 Regulation of IP3R Protein—**MMC grown in Dulbecco’s modified Eagle’s medium (DMEM) (Life Technologies, Inc.) with 10% fetal calf serum were harvested and plated onto 100-mm dishes with growth media. After reaching 80% confluence, cells were incubated in serum-free DMEM for 24 h. During the subsequent final 8 h of incubation, the cells were treated with TGF-β1 (10 ng/ml) for the last 1, 2, 4, and 8 h, washed with PBS three times, and harvested in lysis buffer that contained 50 mM Tris-HCl (pH 7.2), 150 mM NaCl, 1% (w/v) Triton X-100, 1 mM EDTA, 1 mM PMSF, and 5 μg/ml each of aprotinin and leupeptin. All samples, including the control samples, were harvested after the same overall duration of incubation. Protein concentrations of samples were quantitated, and equal amounts of protein were run on a 7% SDS-PAGE gel, transferred to nitrocellulose, and immunoblotted with an antibody raised to the C terminus of the type I IP3R from brain (26). The primary antibody was then removed, and the membrane was incubated with horseradish peroxidase-conjugated secondary antibody. Immunoactive bands were detected using enhanced chemiluminescence (Amersham). Densitometric analysis of scanned images was performed on a Macintosh 7600/132 computer using the public domain NIH Image program. Membranes in control samples were assigned a relative value of 100%.

**Polymerase Chain Reaction-based Analysis of Type I IP3R in MMC and Regulation of Type I IP3R mRNA by TGF-β1—**First strand cDNA was prepared from total RNA isolated from mouse cerebellum, kidney, and MMC using Moloney murine leukemia virus reverse transcriptase (Promega) and oligo(dT) primer. Polymerase chain reaction (PCR) was performed using specific primers for the type I IP3R isoform (5′-CGT GGA TGT TCT ACA CAG ACC AG-3′) and (5′-TTG GAA CTT GAT GAA GAC A-3′) (13). These primers are on either side of the S1′—splice domain of the type I IP3R (13). Each reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 3 mM MgCl2, 0.5 mM each of aprotinin and leupeptin. The first strand cDNA products were inocubated with 1 μl of the antibody to the type I IP3R. The PCR product (see above) from MMC on a low melt agarose gel and then incubated with various agonists for 15 min. The cells were centrifuged and solubilized in lysis buffer, and the extracts were obtained were immunoprecipitated with IP3R antibody as described above. The immunoprecipitates were washed three times in a phosphorylation buffer that contained 120 mM KCl, 50 mM Tris-HCl (pH 7.2), 0.1% Triton X-100 (w/v), 0.3 mM MgCl2, 0.5 mM PMSF, and 10 μg/ml each of aprotinin and leupeptin. Aliquots of protein A-Sepharose beads were incubated in 50 μl of the phosphorylation buffer containing 100 units/ml of the catalytic subunit of PKA and 1 μCi of [γ-32P]ATP (3000 Ci/mmol). The immunoprecipitates were phosphorylated for 15 min at 30 °C, and the reaction was terminated by washing the protein A-Sepharose beads three times with phosphorylation buffer containing 1 mM unlabeled MgATP. The immunoprecipitated proteins were precipitated on 5% SDS-PAGE gels and transferred to nitrocellulose, which was then autoradiographed. The membrane was later immunoblotted to locate the receptor. In all experiments the IP3R was the only phosphorylated band above the prestained myosin molecular mass marker (200 kDa).

**In Vitro Kinase Assay with IP3R Peptide—**The peptides RPSGRESSTSFFGNP and ARDSVLAAS, which include the major phosphorylation site for PKA (serine 1755 and serine 1589) (14, 15) were selected for in vitro kinase assays. MMC incubated in serum-free DMEM were treated with agonists for various periods of time, washed with PBS, harvested with cold extraction buffer (25 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM β-mercaptoethanol, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 mM PMSF), and homogenized with a Dounce homogenizer. The lysate was centrifuged for 5 min at 4 °C at 14,000 × g, and the supernatant was saved. The protein concentrations of the supernatants were quantitated, and equal amounts of protein were added to a reaction mixture containing 40 mM Tris-HCl, pH 7.4, 20 mM MgCl2, 0.1 mg/ml bovine serum albumin, 200 μM IP3R peptide substrate (RPSGRESSTSFFGNP or ARDSVLAAS), and 3000 Ci/mmol [γ-32P]ATP, and 0.5 mM ATP per reaction. Experiments were performed in parallel with the addition of a PKA inhibitor peptide, TTYADFLASGRTGRENNAH (1 μM) (Promega). The reaction was allowed to proceed for 5 min at 30 °C and then terminated with the addition of 2.5 μl guanidine hydrochloride. 10 μl of sample was spotted onto phosphocellulose filter paper (1 × 1 cm) and washed repeatedly with 1 M NaCl and subsequently with 1 M NaCl in 1% H3PO4. The papers were then dried in an oven and placed in scintillation vials for radioactive counting. The stoichiometry of phosphorylation was assessed from the specific activity of [32P]ATP and from the amount of peptide used. The concentration of the peptide substrate and the duration of the in vitro kinase reaction were varied to define the concentration and time dependence of peptide phosphorylation.

**RESULTS**

**Down-regulation of Type I IP3R Protein by TGF-β1—**An antibody raised to the C terminus of the rat brain type I IP3R recognized a 240-kDa polypeptide from the SV40-transformed MMC (Fig. 1A). The same sized band was noted from protein derived from mouse and rat cerebellum (data not shown). The
Mesangial Cells and Mouse Kidney Only Express the SII Form of the IP₃R—It has been previously demonstrated that the IP₃R contains the long form (insertion of the SII segment) of the type I IP₃R, whereas peripheral tissues may contain exclusively the alternatively spliced short form (SII−) or both the SII− and SII+ of the type I IP₃R (13, 14). To evaluate the length of the IP₃R in mesangial cells and mouse kidney, we chose primers that surround the SII domain of the IP₃R cDNA (13). Fig. 4 shows that mesangial cells and kidneys from mice only express a 580-bp DNA segment, whereas cerebellum cDNA expresses a 700-bp segment. As described previously (13), the cerebellum fragment corresponds to the SII− of the IP₃R, and the 580-bp fragment corresponds to the SII+ of the IP₃R that has the SII segment deleted. This was confirmed by sequencing of the 580-bp DNA segment.

Phosphorylation of the IP₃R by TGF-β1—It has been previously demonstrated that phosphorylation of the IP₃R may affect its function; therefore, we evaluated whether TGF-β1 could affect the phosphorylation status of the type I IP₃R. MMC labeled with [³²P]orthophosphate were treated with TGF-β1 for variable times, and the IP₃R was immunoprecipitated and resolved by SDS-PAGE. A time course demonstrating the peak effect of TGF-β1 on phosphorylation of the IP₃R is shown in Fig. 5A. Quantitative analysis demonstrates that phosphorylation was increased by 2-fold at 15 min, 5-fold at 30 min, and 4-fold at 60 min of TGF-β1 treatment (Fig. 5B). As shown in Fig. 6, TGF-β1 and forskolin treatment for 15 min enhanced phosphorylation of the IP₃R to a similar degree.

Back Phosphorylation of the Type I IP₃R by Protein Kinase A—Given that the IP₃R has been previously demonstrated to be phosphorylated by PKA (15, 17), and based on our prior data that cyclic nucleotide dependent kinases may mediate TGF-β1 inhibition of [Ca²⁺] mobilization (10), we asked if PKA might be playing a role in TGF-β1 phosphorylation of the IP₃R. Triton X-100 extracts of unlabeled control or TGF-β1-treated mesan-
gial cells were immunoprecipitated, and the immunoprecipitates were phosphorylated in vitro after incubation with [\(32P\)]ATP and the catalytic subunit of PKA (Fig. 7A, upper panel). The major polypeptide phosphorylated in the immunoprecipitates could be shown to be IP3R by immunoblotting (Fig. 7A, lower panel). The enhanced phosphorylation of the protein in TGF-\(\beta\)1-treated mesangial cells in vivo markedly lowered the incorporation of \(32P\) in the in vitro phosphorylation assay.

Quantitative analysis demonstrates that the degree of in vitro phosphorylation of the IP3R by PKA was decreased to 34% of control with 15 min of TGF-\(\beta\)1 treatment (Fig. 7B). This experiment suggests that TGF-\(\beta\)1 stimulates phosphorylation of the IP3R at sites that are phosphorylated by protein kinase A.

**FIG. 3.** Effects of TGF-\(\beta\)1 on type I IP3R mRNA expression in MMC. Shown is a representative Northern blot of poly(A) mRNA from MMC (3 \(\mu\)g) treated with TGF-\(\beta\)1 (10 ng/ml) for 1 and 4 h and hybridized with a radioactive 580-bp probe for the type I IP3R (A). The blot was stripped and reprobed with \(\beta\)-actin cDNA to standardize the amount of RNA loaded. Panel B shows the densitometric quantitation of IP3R mRNA/\(\beta\)-actin mRNA expressed relative to the control. Data shown are the mean \(\pm\) S.E. of band intensities from three separate experiments. *, \(p < 0.01\) versus control.

**FIG. 4.** PCR products from mouse cerebellum, kidney, and mesangial cell cDNAs. RNA isolated from mouse tissues and MMC was reverse transcribed to cDNA, and PCR was performed using primers flanking the SII insertion site. The location of the molecular size markers for 700 and 500 base pairs are indicated.

**FIG. 5.** Time course of phosphorylation of type I IP3R by TGF-\(\beta\)1 in MMC. MMC labeled with [\(32P\)]orthophosphate were incubated with TGF-\(\beta\)1 (10 ng/ml) for the designated time periods (lanes 3–8). After washing, the cells were solubilized with Triton X-100, and the IP3R was immunoprecipitated as described under “Experimental Procedures.” The immunoprecipitates were analyzed on 5% SDS-PAGE, and the polypeptides were transferred to nitrocellulose. Panel A shows the autoradiogram of a representative experiment. Panel B shows the histogram of mean \(\pm\) S.E. of data derived from three separate experiments. *, \(p < 0.01\) versus control.

**FIG. 6.** Phosphorylation of the type I IP3R by TGF-\(\beta\)1 and forskolin. MMC labeled with [\(32P\)]orthophosphate were incubated with TGF-\(\beta\)1 (10 ng/ml, 15 min) or forskolin (10 \(\mu\)M, 15 min). Immunoprecipitated IP3R was analyzed on 5% SDS-PAGE, transferred to nitrocellulose, and exposed to autoradiography.
phosphorylated peptide (Fig. 8B). Prolonged incubation (>15 min) decreased kinase activity, possibly due to endogenous phosphatase activity present in the lysate. TGF-β1 treatment for 15 min demonstrated enhanced kinase activity at all concentrations of the peptide and at all durations of the *in vitro* kinase reaction. Varying the concentration and time of the kinase reaction of the peptide ARRDSVLAAS gave similar relationships (data not shown) as noted for the peptide RPSGRRESLTSFGNP. For the subsequent experiments, the concentration of the peptide was 200 μM, and the duration of the kinase reaction was 5 min. TGF-β1 treatment of MMC for 5 and 15 min stimulated phosphorylation of the peptide RPSGRRESLTSFGNP by 150 and 158% of control values, respectively (Table I and Fig. 9). Forskolin treatment resulted in slightly greater phosphorylation (201%) at 5 min but phosphorylation similar to that of TGF-β1 at 15 min (157%). The addition of the peptide inhibitor of PKA (PKI) completely prevented the enhanced phosphorylation of this peptide by both forskolin and TGF-β1 (Fig. 9). Based on the specific activity of [32P]ATP and the amount of IP3R peptide added to the reaction mixture, under control conditions 0.038 ± 0.003 mol of phosphate was incorporated per mol of the IP3R peptide RPSGRRESLTSFGNP. TGF-β1 treatment (10 ng/ml for 15 min) increased the phosphorylation to 0.059 ± 0.001 mol of phosphate/mol of the IP3R peptide RPSGRRESLTSFGNP. Although dilute, crude cell lysates do not appear to contain enough kinase activity to demonstrate stoichiometric phosphorylation on the IP3R peptides, this method does allow for comparison of relative kinase activities from treated and untreated cells.

TGF-β1 treatment of MMC for 5 and 15 min also stimulated phosphorylation of the peptide ARRDSVLAAS by 121 and 123% of control values (Table I and Fig. 10). Forskolin treat-
FGNP (200 (19)) in that carbachol decreased IP3R protein expression due to its potent ability to inhibit the protein expression of the type I IP3R. The presence of several ATTTA sites in the 3′-untranslated region of the type I IP3R is a target of regulation by TGF-β1. Therefore, it is likely that degradation of the protein is also enhanced by TGF-β1, since we have previously found the half-life of type I IP3R protein to be 8–11 h (32).

Our observation that TGF-β1 inhibits type I IP3R expression also demonstrates that factors that do not directly induce a [Ca2+]i flux may affect IP3R expression. Carbachol-induced IP3R degradation has been linked to regulation of functional [Ca2+]i stores (19), although the mechanism underlying this linkage has not been demonstrated. TGF-β1 has not been found to directly affect [Ca2+]i in many cell types evaluated (33, 34), including mesangial cells (10). Therefore, it is likely that TGF-β1 regulation of the IP3R differs from the down-regulation observed with [Ca2+]i-mobilizing agonists.

The implication of decreased IP3R protein expression is that [Ca2+]i mobilization would be impaired, as demonstrated previously (18) in neuroblastoma cells. Theoretically, the TGF-β1-treated cell should be less sensitive to mobilize [Ca2+]i, when exposed to any agonist that stimulates IP3R-mediated [Ca2+]i mobilization via the IP3R. Thus, our findings may explain the previous observations that long term exposure (>1 h) to TGF-β1 would inhibit [Ca2+]i mobilization by isoproterenol (8) and PDGF.2

Short term (15–30 min) treatment of TGF-β1 in mesangial and smooth muscle cells are also sufficient to block agonist-induced [Ca2+]i mobilization (9, 10). This effect would not be explained by decreased expression of type I IP3R, since it requires at least 1 h of exposure to TGF-β. The short term effect may be mediated via TGF-β1-induced phosphorylation of the IP3R. It has been demonstrated that phosphorylation of the IP3R may affect its ability to release [Ca2+]i, upon exposure to IP3 (17). This issue is complex, since there are different lengths of the type I IP3R in neuronal and nonneuronal tissues that may affect the site and consequence of phosphorylation (14). Cerebellar type I IP3R is noted to be in the long form with insertion of an SII domain, which is situated between the two major serines (serines 1589 and 1755) that are phosphorylated.

TABLE I

| Condition                | RPSGRRELSFTSGNP | ARDSVLAAS |
|--------------------------|------------------|-----------|
| Control                  | 0.94 ± 0.06      | 0.77 ± 0.03 |
| TGF-β1 (5 min)           | 1.40 ± 0.13a     | 0.93 ± 0.03a |
| TGF-β1 (15 min)          | 1.47 ± 0.01a     | 0.94 ± 0.06a |
| Forskolin (5 min)        | 1.56 ± 0.44a     | 1.17 ± 0.19a |
| Forskolin (15 min)       | 1.48 ± 0.22a     | 1.02 ± 0.09a |

a p < 0.05 versus control kinase activity with the corresponding IP3R peptide substrate.

Fig. 9. In vitro kinase assays with IP3R peptide substrate RPSGRRELSFTSGNP. MMC were treated with TGF-β1 (10 ng/ml) for 5 and 15 min or forskolin (10 μM) for 5 and 15 min. In vitro kinase assays were performed as described under “Experimental Procedures” and as noted in Table I. Cell lysate was added to an in vitro reaction mixture that included [γ-32P]ATP and IP3R peptide substrate RPSGRRELSFTSGNP (200 μM) with or without PKI (1 μM). Data are calculated as percentage increase over control, with the control value assigned as 100%, and are presented as mean ± S.E. from three separate experiments. *, p < 0.05 versus control.

Fig. 10. In vitro kinase assays with IP3R peptide substrate ARDSVLAAS. MMC were treated with TGF-β1 (10 ng/ml) for 5 and 15 min or forskolin (10 μM) for 5 and 15 min. In vitro kinase assays were performed as described under “Experimental Procedures” and as noted in Table I. Cell lysate was added to an in vitro reaction mixture that included [γ-32P]ATP and IP3R peptide substrate ARDSVLAAS (200 μM) with or without PKI (1 μM). Data are calculated as percentage increase over control, with the control value assigned as 100%, and are presented as mean ± S.E. from three separate experiments. *, p < 0.05 versus control.

I IP3R CDNA in both the mouse and rat (28, 29) suggests that mRNA stability and/or translation may be subject to regulation (30, 31). Apart from regulation by TGF-β1 of the mRNA for the type I IP3R, it is likely that degradation of the protein is also enhanced by TGF-β1, since we have previously found the half-life of type I IP3R protein to be 8–11 h (32).

Our observation that TGF-β1 inhibits type I IP3R expression also demonstrates that factors that do not directly induce a [Ca2+]i flux may affect IP3R expression. Carbachol-induced IP3R degradation has been linked to regulation of functional [Ca2+]i stores (19), although the mechanism underlying this linkage has not been demonstrated. TGF-β1 has not been found to directly affect [Ca2+]i in many cell types evaluated (33, 34), including mesangial cells (10). Therefore, it is likely that TGF-β1 regulation of the IP3R differs from the down-regulation observed with [Ca2+]i-mobilizing agonists.

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DISCUSSION

The main conclusion based on the results of our study is that the type I IP3R is a target of regulation by TGF-β1. TGF-β1 has a potent ability to inhibit the protein expression of the type I IP3R after 2–4 h of TGF-β1 exposure in glomerular mesangial cells. The decreased protein expression of the type I IP3R may be partly due to diminished synthesis of the type I IP3R as we found a decrease in mRNA levels prior to observing a decrease in protein levels. Our finding that TGF-β1 decreases steady-state IP3R mRNA levels can be explained either by an effect on transcription of the type I IP3R gene or by enhancement of the degradation rate of the mRNA and will require further studies.

Our results differ somewhat from those of Wojcikiewicz et al. (19) in that carbachol decreased IP3R protein expression due mainly to enhanced degradation of the IP3R in human neuroblastoma cells. However, this group also reported a decrease in mRNA levels after 3 h of exposure to carbachol, which may have contributed to the decreased protein levels. The presence of several ATTTA sites in the 3′-untranslated region of the type I IP3R mRNA levels after 3 h of exposure to carbachol, which may have contributed to the decreased protein levels. The presence of several ATTTA sites in the 3′-untranslated region of the type I IP3R.
by PKA. Using cerebellar derived IP₃R in liposomes, Cameron et al. (16) recently demonstrated that PKA-induced phosphorylation impairs IP₃-mediated [Ca²⁺]ᵢ, whereas protein kinase C-induced phosphorylation of the cerebellar IP₃R enhances IP₃-mediated [Ca²⁺]ᵢ release. PKA-induced phosphorylation of the purified nonneuronal short form of the type I IP₃R primarily occurs on serine 1589 and on serine 1755 (14). Our studies by PCR determined that, similar to other peripheral tissues previously examined (13, 14), mesangial cells and kidney tissue only contain the SII– form of the receptor. The functional result of nonneuronal PKA-induced IP₃R phosphorylation remains unclear. Our in vitro kinase assays demonstrate that forskolin treatment of mesangial cells induces PKA-mediated phosphorylation of both IP₃R peptides that contain serine 1589 and serine 1755. Thus, both sites are potential phosphorylation sites on the nonneuronal IP₃R by PKA. TGF-β1 treatment also resulted in significantly increased phosphorylation of both peptides, although the degree of phosphorylation on the peptide containing serine 1755 was twice as much as the peptide containing serine 1589. It should be noted that prior studies evaluating the site of IP₃R phosphorylation employed purified kinases and purified IP₃R. Our findings employed mesangial cell-derived kinase preparations that were activated by agonists in the intact cell. Our studies do not exclude the possibility that other sites on the type I IP₃R may also be phosphorylated by TGF-β1, which may affect its function.

The interaction of TGF-β with the type I IP₃R probably leads to important modulatory influences on glomerular mesangial cells in vitro. Chronic glomerular overexpression of mesangial-β1 has been demonstrated in experimental diabetic kidney disease, experimental glomerulonephritis, and puromycin-induced nephrosis (1). In experimental diabetes, other factors including PDGF-BB, fibroblast growth factor, endothelin, and angiotensin II are also known to be up-regulated (6, 35). The diabetic kidney demonstrates chronic vasodilatation of the afferent arteriole, mesangial cell stretching, and glomerular hypertrophy without a great degree of mesangial cell proliferation. Theoretically, TGF-β-induced IP₃R down-regulation may impair angiotensin- and endothelin-induced smooth muscle cell and mesangial cell contraction and impair PDGF- and fibroblast growth factor-induced mesangial cell proliferation. Whether this property of TGF-β would be beneficial or deleterious in contributing to chronic renal disease progression remains to be investigated.
