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Diabetes-induced overexpression of endothelin-1 and endothelin receptors in the rat renal cortex is mediated via poly(ADP-ribose) polymerase activation

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ABSTRACT

We hypothesize that poly (ADP-ribosyl)ation, that is, poly (ADP-ribose) polymerase (PARP)-dependent transfer of ADP-ribose moieties from NAD to nuclear proteins, plays a role in diabetic nephropathy. We evaluated whether PARP activation is present and whether two unrelated PARP inhibitors, 3-aminobenzamide (ABA) and 1,5-isoquinolinediol (ISO), counteract overexpression of endothelin-1 (ET-1) and ET receptors in the renal cortex in short-term diabetes. The studies were performed in control rats and streptozotocin-diabetic rats treated with/without ABA or ISO (30 and 3 mg*kg^{-1}*day^{-1}, intraperitoneally, for 2 weeks after 2 weeks of diabetes). Poly (ADP-ribose) immunoreactivity was increased in tubuli, but not glomeruli, of diabetic rats and this increase was corrected by ISO, whereas ABA had a weaker effect. ET-1 concentration (ELISA) was increased in diabetic rats, and this elevation was blunted by ISO. ET-1, ET(A), and ET(B) mRNA (ribonuclease protection assay), but not ET-3 mRNA (RT/PCR), abundance was increased in diabetic rats, and three variables were, at least, partially corrected by ISO. ABA produced a trend towards normalization of ET-1 concentration and ET-1, ET(A), and ET(B) mRNA abundance, but the differences with untreated diabetic group were not significant. Poly(ADP-ribosyl)ation is involved in diabetes-induced renal overexpression of ET-1 and ET receptors. PARP inhibitors could provide a novel therapeutic approach for diabetic complications including nephropathy, and other diseases that involve the endothelin system.

Key words: 3-aminobenzamide • diabetic nephropathy • 1,5-isoquinolinediol • poly(ADP-ribosyl)ation • streptozotocin-diabetic rat

Hyperglycemia causes diabetic nephropathy through several mechanisms among which increased aldose reductase activity (1), non-enzymatic glycation/glycoxidation (2), and activation of protein kinase C (3) are the best studied. All three mechanisms contribute to enhanced oxidative stress (4–8), that is, increased production of reactive oxygen species (ROS) combined with down-regulation or insufficient up-regulation of antioxidant defense mechanisms. Our recent study (9) revealed that enhanced oxidative stress is present in the kidney as early as three weeks after induction of streptozotocin diabetes. Oxidative stress affects all three compartments of the renal cortex, that is, glomeruli (10, 11), tubulo-interstitium (12), and vasculature (13) and contributes to mesangial expansion of extracellular matrix (14, 15) and other early and late events of diabetic nephropathy, that is, increased glomerular filtration rate (16), urinary albumin excretion and proteinuria (14, 15, 17), glomerulosclerosis (14, 18), and tubulo-interstitial fibrosis (18). At least, some of these effects could be mediated through the endothelin system. Endothelin-1 (ET-1) is a potent vasoconstrictor peptide that has multiple signal transduction, metabolic and pathophysiological effects (19). ET-1 overexpression is known 1) to be present in glomeruli (20), tubuli (21), and vasculature (22) of diabetic rats; and 2) to contribute to development of hypertension, proteinuria, albuminuria, tubulo-interstitial inflammation (21) and, at a later stage, mesangial expansion (23). ET-1 mRNA abundance increases with the progression of diabetic nephropathy (24). Antioxidants decrease ET-1 production in the renal cortex of diabetic rats, whereas hydrogen peroxide or ROS generation by xanthine/xanthine oxidase increase ET-1 mRNA and peptide expression (20).

The question of *how* ROS interfere with the endothelin system remains open. Of interest is the fact that diabetes-induced ET-1 overexpression is counteracted by both antioxidants (20) and protein kinase C inhibitors (25). High glucose-induced protein kinase C activation is known to contribute to oxidative stress through phosphorylation (activation) of NADH oxidase, the superoxide generating enzyme (8). In turn, oxidative stress further increases protein kinase C activity via poly(ADP-ribose) polymerase (PARP-1, EC 2.4.2.30) activation (26). Upon binding to the sites of ROS-induced DNA single-strand breakage, PARP cleaves nicotinamide adenine dinucleotide (NAD) with the formation of nicotinamide and (ADP-ribose) residues, which are attached to nuclear proteins and to PARP itself with formation of poly(ADP-ribose) (27, 28). Thus, poly(ADP-ribosyl)ation depletes NAD required for the glyceraldehyde 3-phosphate dehydrogenase reaction of glycolysis, and diverts the glycolytic flux towards the formation of α-glycerophosphate and diacylglycerol, the activator of protein kinase C. The potent and specific PARP inhibitor, N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide HCI (PJ34, 29) blunts high glucose-induced protein kinase C activation in aortic endothelial cells (26), and such inhibition is likely to affect the endothelin system (25). It has also been reported that hyperglycemia-induced ET-1 production in endothelial cells is mediated via the redox-sensitive transcription factor, nuclear factor- kappaB (NF-κB) (30), activation of which is 1) persistent in diabetes (31) and 2) PARP-dependent (32–34).

Thus, current knowledge suggests that PARP is an important component in the cascade, which includes oxidative stress, NF-κB and protein kinase C activation, and ET-1 overexpression, and thus can play an important role in diabetic nephropathy. The present study was designed to assess whether 1) PARP activation is present and 2) whether two structurally unrelated PARP inhibitors, 3-aminobenzamide (ABA) and 1,5-isoquinolinediol (ISO), counteract overexpression of ET-1 and ET (A) and (B) receptors, in the renal cortex of rats with short-term diabetes.

MATERIALS AND METHODS

The experiments were performed in accordance with regulations specified by The Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23) and the University of Michigan Protocol for Animal Studies.

Animals

Male Wistar rats (Charles River, Wilmington, MA), initial body weight 200–230 g, were fed a standard rat chow diet (ICN Biomedicals, Cleveland, OH) and had *ad libitum* access to water. Diabetes was induced by a single injection of streptozotocin (55 mg/kg body weight, i.p.). Blood samples for measurements of glucose were taken from the tail vein ~ 48 h after streptozotocin injection and the day before the rats were killed. Rats with blood glucose of 13.9 mmol/l or more were considered diabetic. The experimental groups included control and diabetic rats treated with or without ABA or ISO at the doses of 30 $mg^* kg^{-1} {}^* d^{-1}$ and 3 $mg^* kg^{-1} {}^* d^{-1}$, respectively, given i.p., for 2 weeks after first 2 weeks without treatment. Thus, the duration of diabetes and the whole experiment in our study was 4 weeks. The *intervention* study was designed to avoid potential regeneration of pancreatic β-cells and restoration of normoglycemia or alleviation of hyperglycemia, which could occur if PARP inhibitor administration was started shortly after induction of diabetes (27).

Reagents

Unless otherwise stated, all chemicals were of reagent-grade quality and were purchased from Sigma Chemical Co., St. Louis, MO. Methanol (HPLC grade), perchloric acid, hydrochloric acid, and sodium hydroxide were obtained from Fisher Scientific, Pittsburgh, PA. Ethyl alcohol (200 proof dehydrated alcohol, U.S.P. punctilious) was purchased from McCormick Distilling Co., Weston, MO. T7 and T3 RNA polymerases were obtained from Roche Molecular Biochemicals, Indianapolis, IN. [α^{32}P]-uridine triphosphate (800 Cu/mmol) was purchased from NEN Life Science Products, Arlington, IL. The ultra-pure guanidine isothiocyanate solution was purchased from Gibco/BRL, Gaithersburg, MD. The vials containing 15 ml of 4% w/v formaldehyde were purchased from Sigma, St. Louis, MO. Primary mouse monoclonal anti-poly(ADP-ribose) antibody was purchased from Alexis, San Diego, CA. The diaminobenzidine (DAB) kit was purchased from Pierce, Rockford, IL, and biotinylated goat anti-mouse immunoglobulin and horseradish peroxidase-conjugated avidin from Vector Laboratories, Burlingame, CA.

Experimental procedure.

Rats were sedated with carbon dioxide and immediately killed by cervical dislocation. The kidneys were rapidly dissected. One kidney was frozen in liquid nitrogen and later used for ET-1 concentration measurements or RNA isolation, and another one was immersed in formaldehyde vials for subsequent paraffin embedding and sectioning for assessment of poly(ADP-ribose) immunoreactivity by immunocytochemistry.

Individual measurements

ET-1 ELISA

Renal cortex material (1 g) was homogenized in 8 ml 0.1 M phosphate buffer, pH 7.5, containing 0.9% NaCI, 0.1% bovine serum albumin, 0.1% Tween 20, and 0.01% thimerosal. ET-1 measurements have been performed in accordance with the manufacturer's instructions.

RNA isolation

Total RNA was extracted from the kidney by using the acid guanidium-phenol-chloroform extraction method as we have described previously (35). Kidneys were minced on ice and homogenized in a polytron tissue homogenizer with 4 ml of guanidine isothiocyanate solution. Sequentially, 0.4 ml of 2 M sodium acetate, pH 4.0, 4 ml of phenol (water saturated), and 0.8 ml of chloroform-isoamyl alcohol mixture (49:1) were added to homogenate, with thorough mixing after the addition of each reagent. RNA was precipitated with equal volume of 2-propanol. RNA pellets were washed with 75% ethanol and dissolved in nuclease-free water.

Ribonuclease protection assays of ET-1 and ET(A) and ET(B) receptors

The plasmids for synthesis of rat ET-1 and endothelin receptors probes (36) and rat 18S probe (35) were described previously. Synthesis of radiolabelled probes for ribonuclease protection assay was performed according to the Roche protocol by using T7 or T3 RNA polymerase and [α-^{32}P]-UTP. For ribonuclease protection assays, water solutions of total RNA were dried under vacuum and dissolved in 25 µl of 80% formamide hybridization buffer containing labeled probes. Samples were preincubated for 5 min at 85°C and then incubated for 16 h at 45°C as described previously (37). The extracted, protected probe fragments were run on a 6% polyacrylamide sequencing gel in 1´ Tris-borate-EDTA buffer at 50 mA for 2 h. The gels were then dried and exposed to X-ray film (Hyperfilm MP, Amersham, Arlington Heights, IL) at –70°C. mRNA expression was quantified by using the storage phosphor technology (Molecular Dynamics, Sunnyvale, CA). The intensities of mRNA bands were normalized to 18S ribosomal RNA.

RT/PCR assay of ET-3 mRNA

ET-3 mRNA abundance was analyzed by RT/PCR. First-strand cDNA synthesis was performed at 42°C for 20 min by using 2 µg total RNA from mouse lung in a 20 µl reaction mixture containing 50 mM Tris/HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 20 µM dNTP (a mixture of equal amounts of dATP, dGTP, dCTP and dTTP), 1 µM oligo(dT), and 200 U superscript reverse transcriptase (RT; Gibco/BRL, Gaithersburg, MD). The ET-3 fragment was amplified by using forward primer (5'-TCGGGCTCACAGTGACCTCC-3') corresponding to 197–216, and reverse primer (5'-GTGGTTGGACAGTCCATAGG-3') corresponding to 435–416 sequences of the rat ET-3 gene (GenBank™ accession number S39779). Amplified PCR products were isolated by agarose gel electrophoresis and stained with ethidium bromide.The intensities of ET-3 mRNA bands were normalized to 18S rRNA.

Poly(ADP-ribose) immunoreactivity

All immunohistochemical samples were coded and examined by a single investigator in a blinded fashion. Paraffin-embedded 4-μm-thick renal cortex sections were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol followed by a 5-min incubation in phosphate-buffered saline (PBS). Sections were treated with 0.3% hydrogen peroxide for 15 min to block endogenous peroxidase activity and then were rinsed briefly in PBS. Non-specific binding was blocked by incubating the slides for 1 h in 0.25% Triton/PBS containing 2% horse serum. To detect poly(ADP) ribose, a routine histochemical procedure was applied as previously described (38), with minor modifications as follows. Mouse monoclonal anti-poly (ADP-ribose) antibody and isotype-matched control antibody were applied in a dilution of 1:400 for 1 h at room temperature. After extensive washing (3 times, 10 min) with 0.25% Triton/PBS, immunoreactivity was detected with a biotinylated horse anti-mouse secondary antibody and the avidin-biotin-peroxidase complex both supplied in the Vector Elite kit. Color was developed by using the DAB substrate kit. Sections were then briefly rinsed in Tris/Saline, pH 7.6, and incubated in Tris/Cobalt, pH 7.2, for 2 min. Sections were then counterstained with nuclear fast red, dehydrated, and mounted in permount. Microphotographs were taken with a Zeiss Axiolab (Axiolab, Charlotte, VT) microscope equipped with a Fuji HC-300C digital camera.

Statistical analysis

The results are expressed as mean ± SEM. Data were subjected to equality of variance *F*-test, and then to log transformation, if necessary, before one-way analysis of variance. When overall significance ($P<0.05$) was attained, individual between-group comparisons were made by using the Student-Newman-Keuls multiple range test. When between-group variance differences could not be normalized by log transformation, the data were analyzed by the non-parametric Kruskal-Wallis one-way analysis of variance, followed by the Fisher's PLSD test for multiple comparisons.

RESULTS

The final body weights (Table 1) were lower in diabetic rats than in the control group ($P<0.01$) and were slightly, but significantly, lower in ABA-treated diabetic rats compared with the untreated group ($P<0.01$). No differences were found between ISO-treated diabetic rats and the untreated group. Both agents were well tolerated, and no side effects were observed. A 2-week administration of ABA and ISO started after first 2 weeks without treatment did not result in reversal or amelioration of hyperglycemia in diabetic rats. Blood glucose concentrations were approximately fivefold higher in untreated diabetic rats, and diabetic rats treated with ABA or ISO compared with the non-diabetic control group ($P<0.01$ for all three comparisons).

Poly(ADP-ribose) staining in the tubuli of the renal cortex was more intense in diabetic rats than in the control group (Fig. 1A and B). The diabetes-induced increase in poly(ADP-ribose) immunoreactivity was slighly blunted, but not corrected, by ABA (Fig. 1C), and was corrected by ISO (Fig. 1D). Poly(ADP-ribose) immunoreactivity in the glomeruli was indistinguishable among the groups (Fig. 1E–H).

Renal cortex ET-1 concentration was increased by 75% in diabetic rats compared with the control group (1658±197 vs. 947±133 pg/g renal cortex, $P<0.05$), and this increase was normalized by ISO (810±158 pg/g, $P<0.05$ vs. diabetic group). ABA treatment produced a trend towards a decrease of this concentration (1254±229 pg/g), but the difference with the untreated diabetic group did not achieve statistical significance.

Renal ET-1 mRNA expression was increased by 47% in diabetic rats compared with controls ($P<0.01$, Fig. 2A). This increase was partially corrected by ISO, to 110 ± 4% of the control value ($P<0.01$ vs. diabetic group). ET-1 mRNA abundance tended to decrease in diabetic rats treated with ABA, but the difference with the untreated group did not achieve statistical significance.

Renal ET-3 mRNA abundance was not affected by diabetes or PARP inhibitor treatment (Fig. 2B).

Renal ET(A) mRNA expression was increased by 31% in diabetic rats compared with controls ($P<0.01$, Fig. 2C). This increase was partially corrected by ISO, to 108 ± 4% of the control value ($P<0.01$ vs. diabetic group). ET(A) mRNA abundance tended to decrease in the ABA-treated diabetic rats, but the difference with untreated diabetic group did not achieve statistical significance.

Renal ET(B) mRNA expression was increased by 50% in diabetic rats compared with controls ($P<0.01$, Fig. 2D). This increase was partially corrected by ISO, to 116± 4% of the control value ($P<0.01$ vs. diabetic group). ET(B) mRNA abundance was slightly lower in the ABA-treated diabetic rats, but the difference with untreated group did not achieve statistical significance.

DISCUSSION

Numerous findings indicate that PARP activation is an important step in various pathological states associated with oxidative stress (27). Enhanced poly(ADP-ribosyl)ation has been described in experimental models of cardiovascular and inflammatory diseases, cancer, and, recently, diabetes mellitus (27, 29, 39–41). The pancreatic β-cell necrosis caused by high doses of diabetogenic agents, streptozotocin, and alloxan is prevented by the PARP inhibitors, nicotinamide, and ABA, and does not occur in PARP-knockout (PARP –/–) mice (27). Furthermore, the "slow" cytokine-mediated autoimmune destruction of β-cells in the murine multiple-low-dose-STZ-diabetic model is also preventable by PARP inhibitors, which implicates PARP activation in the pathogenesis of Type 1 (insulin-dependent) diabetes mellitus (39). Apart from pancreatic β-cells, enhanced poly(ADP-ribosyl)ation was found to be present in vascular endothelium (29), myocardium(40), peripheral nerve(42), and retina (43) of diabetic animals, as well as skin vessels of diabetic patients (41). This finding thus suggests that PARP activation could be a universal mechanism in the pathogenesis of both macrovascular and microvascular complications of diabetes mellitus. Indeed, PARP inhibition or PARP deficiency have been demonstrated to prevent the development of diabetes-induced endothelial dysfunction (29), myocardial injury (40), and peripheral neuropathy (42).

Our results provide the first evidence of PARP activation in the rat renal cortex very early, that is, 4 weeks, after induction of STZ-diabetes, which temporarily coincides with clearly manifest oxidative stress (9). Of interest, diabetes-induced poly (ADP-ribose) immunoreactivity was

observed in the tubuli, but not glomeruli, of the renal cortex of STZ-diabetic rats. Such localization is in-line with the development of tubulo-interstitial inflammation, but not glomerular lesions in early diabetes (21), as well as with the key role for PARP activation in inflammatory process (27, 44). Taking into consideration that PARP activation is triggered by DNA single-strand breakage (27), our results are also consistent with the 1) presence of DNA single-strand breakage (45); 2) increased apoptotic index, that is, the ratio of bax mRNA/bcl-2 mRNA (46); and 3) overexpression of munc 13-1 and 13-2 genes, known to serve as a diacylglycerol-activated, protein kinase C-independent signaling pathway capable of inducing apoptosis, in tubular epithelium of rats and mice with short-term diabetes (47). Note that, of two PARP inhibitors, only ISO caused a substantial inhibition of poly(ADP-ribosyl)ation in the diabetic kidney. The latter is consistent with different efficacy of the two inhibitors against diabetes-induced changes in the renal endothelin system in the present study, as well as relatively weak PARP-inhibiting capacity of ABA in other reports (48–50). It is important to note that ABA caused an essential normalization of 1) nerve blood flow and conduction deficits (42) and 2) retinal VEGF formation (43) in the same diabetic animals. It is possible that there is regional heterogeneity in tissue uptake or residence time of ABA *in vivo*.

Another important finding in the present study is the demonstration of the major role of PARP activation in diabetes-induced ET-1 overexpression. The increased abundance of ET-1 mRNA and peptide in the renal cortex of diabetic rats in our experiments is in line with other reports (21, 23, 51). It is noteworthy, however, that the values of tissue ET-1 reported to date vary considerably. Our renal ET-1 values are in the range of those reported by four other groups (52–55) who employed different techniques, that is, radioimmunoassay (RIA) or reverse-phase HPLC coupled to RIA. Increased gene expression of both ET(A) and ET(B) receptors in the renal cortex of rats with one-month diabetes in our study is consistent with another study in the rat model (51), as well as increased ET (A) receptor binding in rabbits with short-term diabetes compared with controls (56). ISO treatment caused an essential correction of both ET-1 concentration and ET-1, ET(A) and ET(B) mRNA abundance, whereas ABA caused a trend towards normalization of these variables, consistent with a weaker effect on tubular poly (ADP-ribosyl)ation. These data are consistent with primarily tubular localization of ET-1 in the renal cortex in early diabetes (56). In contrast to the report (51), we did not find any up-regulation of E-3 mRNA in the kidney of rats with 1-month duration of STZ-diabetes.

The role for ET-1 and ET receptors in chronic nephropathies is well established and is supported by findings in 1) ET-1-overexpressing mice that are phenotypically characterized by renal lesions (57, 58), and 2) experimental models of kidney diseases [e.g., hypertension-associated vascular and glomerular fibrosis (59), immune nephritis (60) and diabetes (21, 61)], demonstrating prevention or correction of renal lesions by ET receptor antagonists selectively binding to ET(A) or unselectively to both ET(A) and (B) receptors. The present study suggests that PARP inhibition could be an alternative approach towards the control of the endothelin system and could provide a correction of all three most important variables, that is, ET-1, ET(A) and ET(B) in the diabetic kidney. The role for PARP-1 as a basal transcriptional regulator is well established (27, 29, 32–34, 62–64) and is supported by the DNA chip analysis data revealing marked differences in the gene expression profile between PARP-deficient and wild-type cells (62, 64). In addition to already mentioned NF-κB (32–34), PARP-1 dependent activation of transcription factors induced by stress or inflammatory agents has been demonstrated for activator protein-1 (AP-1); Sp1 transcription factor (SP-1); signal transducer and activator of

transcription 1, 91 kDa (Stat-1); and POU domain, class 2, transcription factor 1 (Oct-1) (64, 65). PARP dependence of AP-1 activation may be particularly relevant for the explanation of our findings, considering that AP-1 is involved in stress-induced ET-1 and ET(B) gene expression (66–68). Previously, it has been reported that, by controlling activation of NF-κB and other transcription factors, PARP regulates expression of numerous NF-κB- or other transcription factor-regulated genes, including those encoding tumor necrosis factor-α, intracellular adhesion molecule-1, P- and E-selectins, integrins, interleukins, inducible nitric-oxide synthase, cyclooxygenase-2, and others (27, 64). The present study suggests that this list can be complemented by the genes encoding ET-1 and ET receptors.

Taking into consideration that ET-1 contributes to the pathophysiology of other diabetic complications, that is, neuropathy (69, 70) and retinopathy (71), and both ET-1 overexpression and PARP activation are present in the diabetic retina (25, 43) and vascular endothelium (27, 29, 30), our findings suggest that PARP inhibition could be useful in eliminating the adverse consequences of ET-1 overexpression, and, in particular, the decrease in retinal and nerve blood flow and inflammation, in diabetic retinopathy and neuropathy.

To summarize, our results indicate that diabetes-induced overexpression of ET-1 and ET(A) and ET (B) receptors in the renal cortex is mediated via PARP activation (Fig. 3) and can be, at least partially, corrected by PARP inhibitors. These data provide the rationale for further studies of potent and specific PARP inhibitors, to prevent or delay diabetic nephropathy as well as other diabetic complications and pathological states associated with ET-1 overexpression.

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Table 1

Final body weights and blood glucose concentrations in control rats and diabetic rats treated with or without ABA or ISO (*n*=30–32).

	Body weight(g)	Blood glucose(mmol/l)
Control	462 ± 7	4.27 ± 0.17
Diabetic	390 ± 24**	20.4 ±1.44**
Diabetic+ABA	343 ± 9**##	20.1 ± 0.50**
Diabetic+ISO	363 ± 8**	20.8 ± 0.39**

**Significantly different from controls (P<0.01, respectively).
##Significantly different from untreated diabetic group (P<0.01).
ABA, 3-aminobenzamide; ISO, 1,5-isoquinolinediol.

Fig. 1

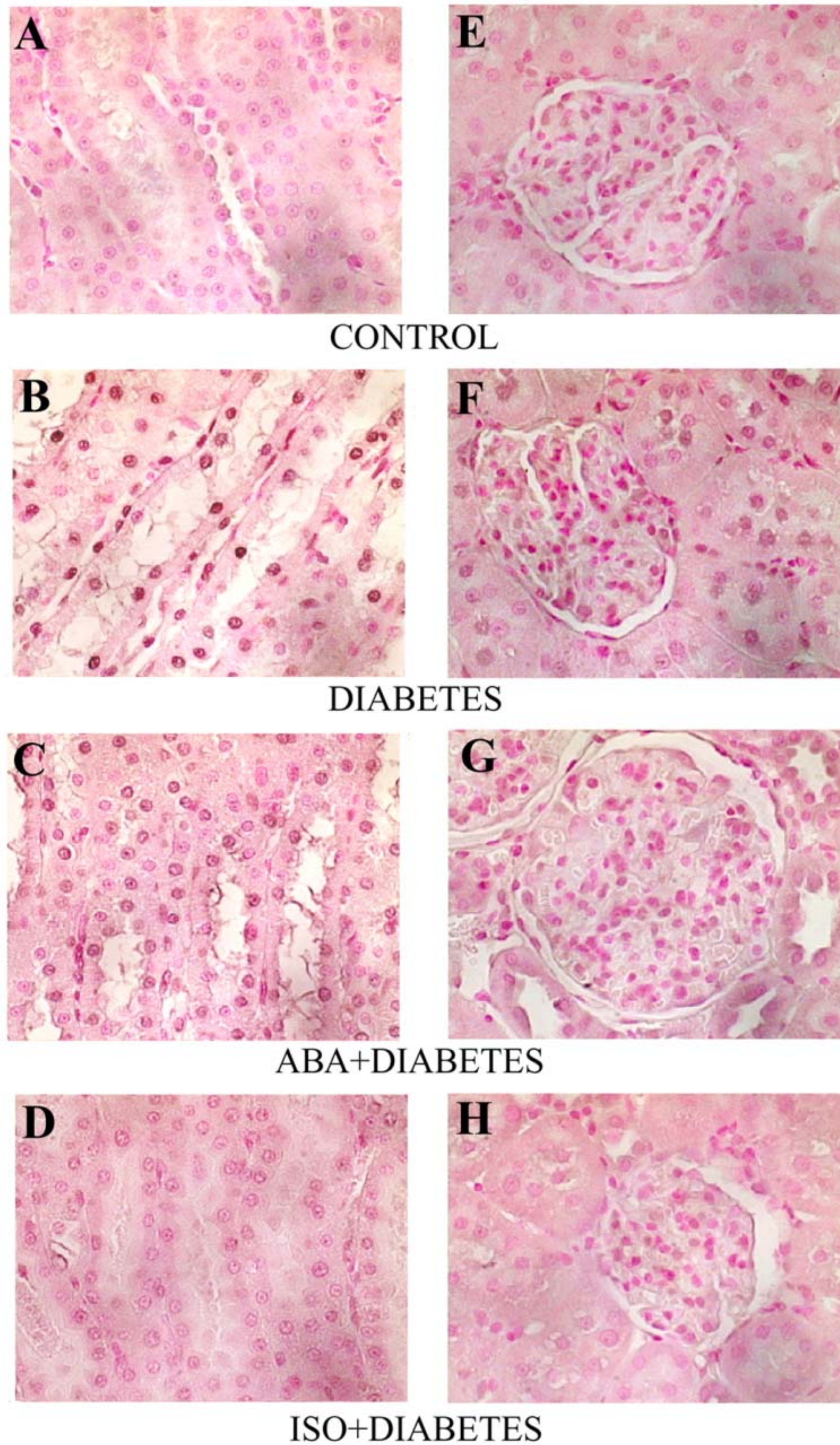

Figure 1. Representative microphotographs of immunocytochemical staining of poly(ADP-ribose) in the tubuli (**A–D**) and glomeruli (**E–H**) of the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*n*=8 per group). Magnification ×400.

Fig. 2

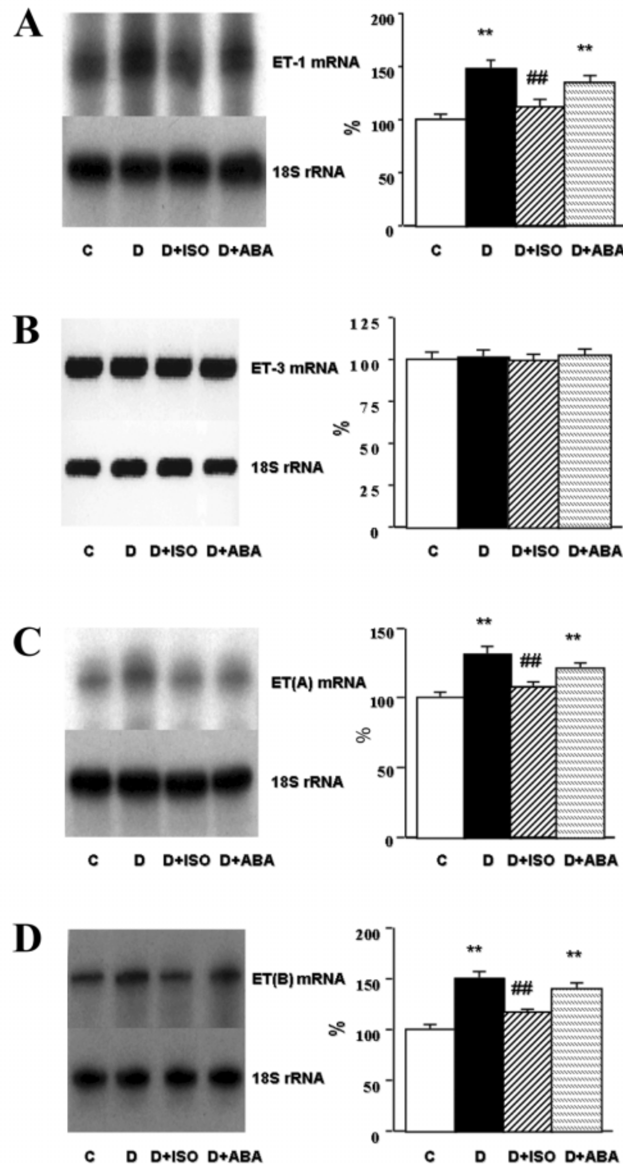

Figure 2. A) Representative polyacrylamide gel analysis obtained in the ribonuclease protection assay of ET-1 and 18 S ribosomal RNA in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*left*). ET-1 mRNA abundance (Mean ± SEM, n=8) in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*right*). Data were normalized to 18S ribosomal RNA. ET-1 mRNA abundance in control rats is taken as 100%. C-control; D-diabetic. $^{**}P < 0.01$ vs. control group; $^{\#\#}P < 0.01$ vs. untreated diabetic group. **B)** Representative agarose gel analysis obtained in the RT/PCR assay of ET-3 and 18 S ribosomal RNA in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*left*). ET-3 mRNA abundance (mean ± SEM, n=8) in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*right*). Data were normalized to 18S ribosomal RNA. ET-1 mRNA abundance in control rats is taken as 100%. C-control; D-diabetic. **C)** Representative polyacrylamide gel analysis obtained in the ribonuclease protection assay of ET(A) and 18 S ribosomal RNA in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*left*). ET(A) mRNA abundance (mean ± SEM, n=8) in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*right*). Data were normalized to 18S ribosomal RNA. ET(A) mRNA abundance in control rats is taken as 100%. C-control; D-diabetic. $^{**}P < 0.01$ vs. control group; $^{\#\#}P < 0.01$ vs. untreated diabetic group. **D)** Representative polyacrylamide gel analysis obtained in the ribonuclease protection assay of ET(B) and 18 S ribosomal RNA in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*left*). ET(B) mRNA abundance (mean ± SEM, n=8) in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (*right*). Data were normalized to 18S ribosomal RNA. ET(B) mRNA abundance in control rats is taken as 100%. C-control; D-diabetic. $^{**}P < 0.01$ vs. control group; $^{\#\#}P < 0.01$ vs. untreated diabetic group.

Fig. 3

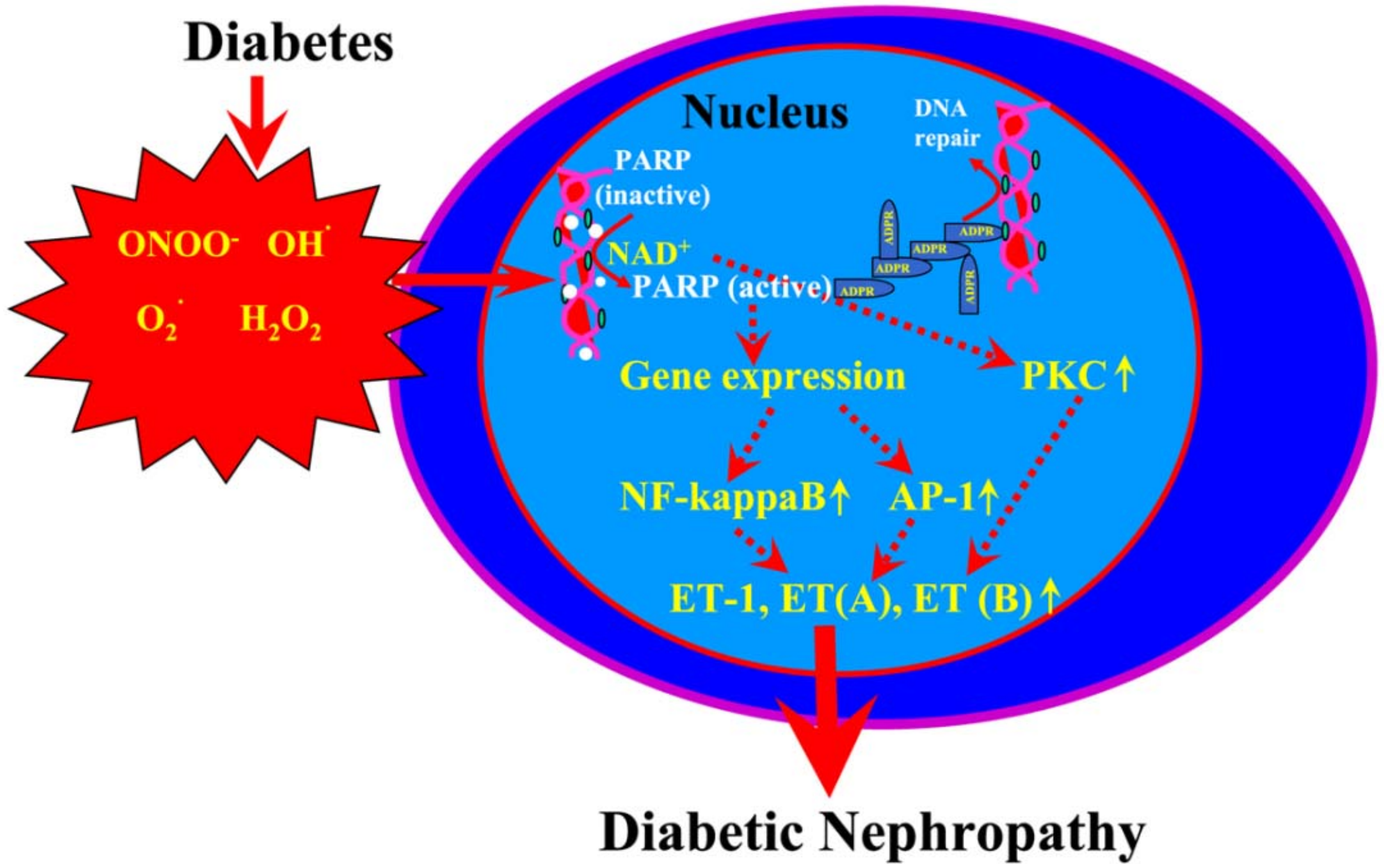

Figure 3. Role for oxidative stress-induced PARP activation in ET-1 and ET receptor overexpression and diabetic nephropathy: potential involvement of NF-κB, AP-1 and protein kinase C. PKC, protein kinase C. ADPR, ADP-ribose.