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

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SPECIFIC AIMS

Oxidative stress is an important event in the pathogenesis of diabetic nephropathy. It affects all three compartments of the renal cortex (glomeruli, tubulo-interstitium, and vasculature) and contributes to mesangial expansion of extracellular matrix, increased glomerular filtration rate, proteinuria, glomerulosclerosis, and tubulo-interstitial fibrosis. 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. Antioxidants decrease ET-1 production in the renal cortex of diabetic rats whereas hydrogen peroxide increases ET-1 mRNA and peptide expression.

The question of *how* ROS interfere with the endothelin system remains open. Recent studies suggest the important role of protein kinase C (PKC) and the redox-sensitive transcription factor nuclear factor κB (NF-κB). Activation of PKC and NF-κB is persistent in diabetes, and is caused by poly(ADP-ribose) polymerase (PARP-1, EC 2.4.2.30) activation. 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 with formation of poly(ADP-ribose). The present study was designed to assess whether 1) PARP activation is present and 2) 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 streptozotocin-induced diabetes.

PRINCIPAL FINDINGS

1. PARP activation is present in the tubuli, but not glomeruli, of the renal cortex of diabetic rats and is corrected by ISO and (to a lesser extent) by ABA

Experiments were performed in control rats and diabetic rats treated with or without the PARP inhibitors ABA or ISO at the doses of 30 $mg \cdot kg^{-1} \cdot day^{-1}$ and 3 $mg \cdot kg^{-1} \cdot day^{-1}$, respectively, given intraperitoneally for 2 wk after 2 wk without treatment. The intervention study was designed to avoid potential regeneration of pancreatic β cells and alleviation of hyperglycemia that could occur if PARP inhibitor administration was started shortly after induction of diabetes. ABA and ISO treatment did not result in reversal or amelioration of hyperglycemia in diabetic rats. Blood glucose concentrations were ~fivefold higher in untreated diabetic rats and diabetic rats treated with ABA or ISO compared with the nondiabetic control group. Poly(ADP-ribose) staining in the tubuli of the renal cortex was more intense in diabetic rats than in the control group (**Fig. 1*A, B***). The diabetes-induced increase in poly-(ADP-ribose) immunoreactivity was slightly blunted, but not corrected, by ABA (Fig. 1*C*) but was corrected by ISO (Fig. 1*D*). Poly(ADP-ribose) immunoreactivity in the renal cortex glomeruli was indistinguishable among the groups (Fig. 1*E–H*).

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.03-0013fje; doi: 10.1096/fj.03-0013fje

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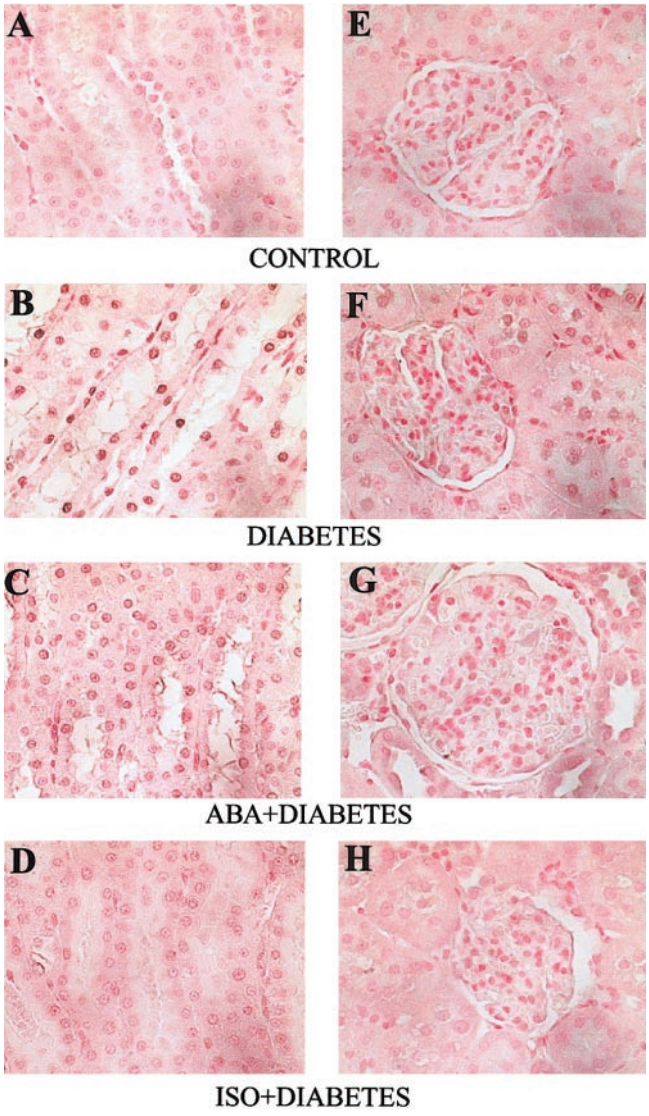

Figure 1. Representative microphotographs of immunocytochemical staining of poly(ADP-ribose) in the tubuli (*A–D*) and glomeruli (*E–H*) of the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (n=8 per group). X 400.

2. ET-1 peptide and mRNA overexpression, but not ET-3 mRNA overexpression, is present in the renal cortex of diabetic rats and is corrected by ISO and (to a lesser extent) by ABA

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); this increase was normalized by ISO (810±158 pg/g, P<0.05 vs. diabetic group). ABA treatment decreased this concentration (1254±229 pg/g) but the difference from the untreated diabetic group did not achieve statistical significance.

Renal cortex ET-1 mRNA expression was increased by 47% in diabetic rats vs. controls (P<0.01, **Fig. 2*A***). 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 cortex ET-3 mRNA abundance was not affected by diabetes or PARP inhibitor treatment (not shown).

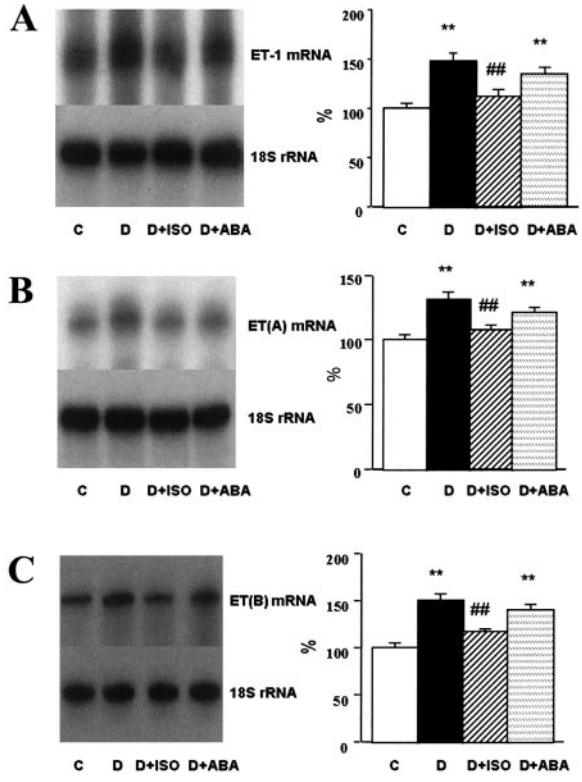

Figure 2. *A)* Representative polyacrylamide gel analysis obtained in the RNase protection assay of ET-1 and 18 S rRNA in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (left). ET-1 mRNA abundance (mean±SE, n=8) in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (right). Data were normalized to 18S rRNA. 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 polyacrylamide gel analysis obtained in the RNase protection assay of ET(A) and 18 S rRNA in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (left). ET(A) mRNA abundance (Mean±SE, 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 rRNA. 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. *C)* Representative polyacrylamide gel analysis obtained in the RNase protection assay of ET(B) and 18 S rRNA in the renal cortex of control rats and diabetic rats treated with or without ABA or ISO (left). ET(B) mRNA abundance (Mean ± SE, 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 rRNA. 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.

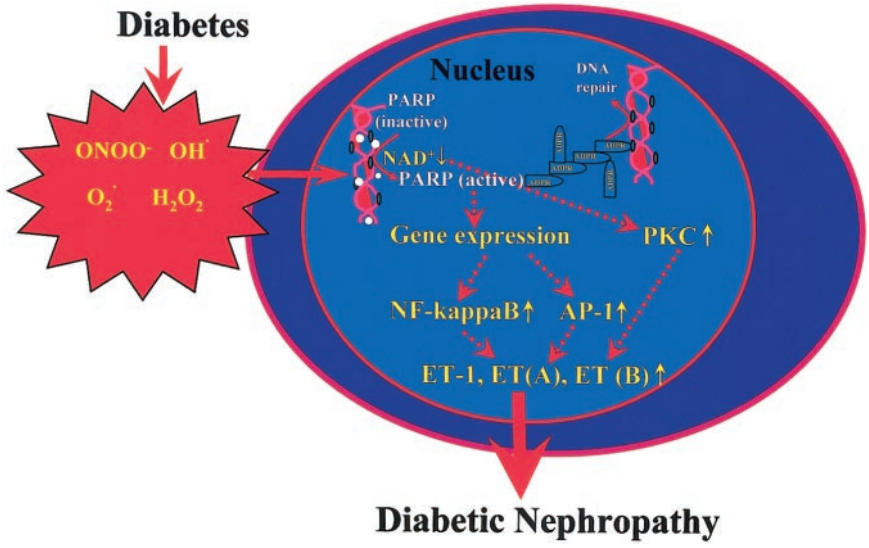

Figure 3. Role for oxidative stress-induced PARP activation in ET-1 and ET receptor overexpression and diabetic nephropathy: potential involvement of NF-κB, AP-1, and protein kinase C (PKC). ADPR, ADP-ribose.

3. ET(A) and ET(B) receptor mRNA overexpression is present in the renal cortex of diabetic rats and is corrected by ISO and (to a lesser extent) by ABA.

Renal ET(A) mRNA expression was increased by 31% in diabetic rats compared with controls ($P<0.01$, Fig. 2*C*). 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. Renal ET(B) mRNA expression was increased by 50% in diabetic rats compared with controls ($P<0.01$, Fig. 2*D*). 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.

CONCLUSIONS

Numerous findings indicate that PARP activation is an important step in various pathological conditions associated with oxidative stress including diabetes. Our results provide the first evidence of PARP activation in the rat renal cortex very early (4 wk) after induction of STZ diabetes.

Another important finding in the present study is the demonstration of the major role of PARP activation in diabetes-induced ET-1, ET(A) and ET(B) overexpression. ISO treatment caused an essential corection of both ET-1 concentration and ET-1, ET(A) and ET(B) mRNA abundance whereas ABA caused a tend towards normalization of these variables, consistent with a weaker effect on tubular poly(ADP-ribosyl)ation.

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, and 2) experimental models of kidney diseases (e.g., hypertension-associated vascular and glomerular fibrosis, immune nephritis and diabetes) 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 to the control of the endothelin system, and could provide a correction of all three most important variables—ET-1, ET(A), and ET(B)—in the diabetic kidney. 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. The present study suggests that this list can be complemented by the genes encoding ET-1 and ET receptors.

To summarize, our results indicate that diabetes-induced overexpression of ET-1, ET(A), and ET(B) receptors in the renal cortex is mediated via PARP activation (**Fig. 3**) and can be corrected by PARP inhibitors. The bidirectional effects of diabetes and PARP inhibition on the endothelin system could be mediated via activation/inhibition of PKC, NF-κB and other transcription factors. 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. FJ