

## Forum Review

# Role of Poly(ADP-Ribose) Polymerase-1 Activation in the Pathogenesis of Diabetic Complications: Endothelial Dysfunction, as a Common Underlying Theme

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### ABSTRACT

Hyperglycemia-induced overproduction of superoxide by mitochondrial electron-transport chain triggers several pathways of injury involved in the pathogenesis of diabetic complications [protein kinase C (PKC), hexosamine and polyol pathway fluxes, advanced glycation end product (AGE) formation] by inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity. Increased oxidative and nitrosative stress activates the nuclear enzyme, poly(ADP-ribose) polymerase-1 (PARP). PARP activation, on the one hand, depletes its substrate, NAD<sup>+</sup>, slowing the rate of glycolysis, electron transport, and ATP formation. On the other hand, it inhibits GAPDH by poly(ADP-ribose)lation. These processes result in acute endothelial dysfunction in diabetic blood vessels, which importantly contributes to the development of various diabetic complications. Accordingly, hyperglycemia-induced activation of PKC isoforms, hexosaminase pathway flux, and AGE formation is prevented by blocking PARP activity. Furthermore, inhibition of PARP protects against diabetic cardiovascular dysfunction in preclinical models. PARP activation is present in microvasculature of human diabetic subjects. The oxidative/nitrosative stress–PARP pathway leads to diabetes-induced endothelial dysfunction, which may be an important underlying mechanism for the pathogenesis of other diabetic complications (cardiomyopathy, nephropathy, neuropathy, and retinopathy). This review focuses on the role of PARP in diabetic complications and the unique therapeutic potential of PARP inhibition in the prevention or reversal of diabetic complications. *Antioxid. Redox Signal.* 7, 1568–1580.

### INTRODUCTION

**P**OLY(ADP-RIBOSE) POLYMERASE (PARP) is a nuclear DNA repair enzyme with multiple regulatory functions (23–25, 38, 49, 55, 85, 95, 96, 99, 114). Overactivation of PARP represents an important mechanism of tissue damage in various pathological conditions associated with oxidative and nitrosative stress, including myocardial reperfusion injury (107, 120), heart transplantation (106), heart failure (70, 71), stroke (31, 45), circulatory shock (42, 68, 69, 89, 93, 98), and autoimmune  $\beta$ -cell destruction associated with diabetes

mellitus (10, 80). Activation of PARP and beneficial effect of various PARP inhibitors have been demonstrated in various forms of endothelial dysfunction, such as those associated with circulatory shock, hypertension, atherosclerosis, pre-eclampsia, and aging (41, 54, 73, 74, 98). Furthermore, recent evidence suggests that activation of PARP importantly contributes to the development of endothelial dysfunction in various experimental models of diabetes and also in humans (33, 72, 91, 102). In addition, it has recently been demonstrated that PARP activation plays a pathogenetic role in diabetic nephropathy, neuropathy, and retinopathy. The follow-

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ing review will discuss the role of PARP activation in the pathogenesis of diabetic complications with special focus on endothelial dysfunction, as a common underlying theme.

## THE PROCESS OF PARP ACTIVATION

Poly(ADP-ribose) polymerase-1 (PARP-1; EC 2.4.2.30) [also known as poly(ADP-ribose) synthetase (PARS) or poly(ADP-ribose) transferase (ADPRT)] is a member of the PARP enzyme family consisting of PARP-1 and an increasing number of additional, recently identified poly(ADP-ribosyl)ating enzymes (minor PARP isoforms). PARP-1, the major PARP isoform, is one of the most abundant proteins in the nucleus. PARP-1 is a 116-kDa protein that consists of three main domains: the N-terminal DNA-binding domain containing two zinc fingers, the automodification domain, and the C-terminal catalytic domain. The primary structure of the enzyme is highly conserved in eukaryotes with the catalytic domain showing the highest degree of homology between different species. The structure and functions of PARP have been the subject of several recent overviews and monographs (95, 96, 114, 118). For the purpose of the current review, it is important to note that PARP-1 is considered the major isoform of PARP in intact cells, and remains commonly termed as "PARP."

PARP-1 plays an important role in multiple physiological functions, as well as in the pathophysiology of many diseases. This has been a subject of several recent reviews and monograph (24, 25, 49, 95, 98). PARP-1 functions as a DNA damage sensor and signaling molecule binding to both single- and double-stranded DNA breaks. Upon binding to damaged DNA (mainly through the second zinc finger domain), PARP-1 forms homodimers and catalyzes the cleavage of nicotinamide adenine dinucleotide (NAD<sup>+</sup>) into nicotinamide and ADP-ribose and uses the latter to synthesize branched nucleic acid-like polymers of poly(ADP-ribose) covalently attached to nuclear acceptor proteins. The size of the branched polymer varies from a few to 200 ADP-ribose units. Due to its high negative charge, covalently attached ADP-ribose polymer dramatically affects the function of target proteins. *In vivo* the auto-poly(ADP-ribosyl)ation represents a major regulatory mechanism for PARP-1, resulting in the down-regulation of the enzyme activity. In addition to PARP-1, histones are also considered as major acceptors of poly(ADP-ribose). Poly(ADP-ribosyl)ation confers negative charge to histones, leading to electrostatic repulsion between DNA and histones. This process has been implicated in chromatin remodeling, DNA repair, and transcriptional regulation. Several transcription factors, DNA replication factors, and signaling molecules [nuclear factor- $\kappa$ B (NF $\kappa$ B), activator protein-1 (AP-1), Oct-1, YY1, TEF-1, DNA-PK, p53] have also been shown to become poly(ADP-ribosyl)ated by PARP-1. The effect of PARP-1 on the function of these proteins is carried out by noncovalent protein-protein interactions and by covalent poly(ADP-ribosyl)ation (for review, see 114).

Poly(ADP-ribosyl)ation is a dynamic process as indicated by the short half-life of the polymer. Two enzymes, poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosyl

protein lyase, are involved in the catabolism of poly(ADP-ribose) with PARG cleaving ribose-ribose bonds of both linear and branched portions of poly(ADP-ribose) and the lyase removing the protein proximal ADP-ribose monomer (23). PARP-1 plays a role in DNA repair and maintenance of genomic integrity (55, 85) and also regulates the expression of various proteins at the transcriptional level. Of special importance is the regulation by PARP-1 of the production of inflammatory mediators such as inducible nitric oxide synthase (iNOS), intercellular adhesion molecule-1 (ICAM-1), and major histocompatibility complex class II (30, 38, 90, 99, 120). NF- $\kappa$ B is a key transcription factor in the regulation of this set of proteins, and PARP has been shown to act as a coactivator in the NF- $\kappa$ B-mediated transcription. Poly(ADP-ribosyl)ation can loosen up the chromatin structure, thereby making genes more accessible for the transcriptional machinery (37, 48, 67, 79, 81, 88).

PARP-1 activation has been proposed to represent a cell elimination pathway whereby severely damaged cells are removed from tissues. PARP-1-mediated cell death occurs in the form of necrosis, which is the least desirable form of cell death. During necrotic cell death, the cellular content is released into the tissue exposing neighboring cells to harmful attacks by proteases and various proinflammatory intracellular factors, and triggering positive feedback pathways of inflammatory tissue injury.

Recently, it has been shown that poly(ADP-ribose) polymer can also serve as an emergency source of energy used by the base excision machinery to synthesize ATP (66). Furthermore, poly(ADP-ribose) may also serve as a signal for protein degradation in oxidatively injured cells (109).

Under pathophysiological conditions, reactive species (such as hydrogen peroxide, hydroxyl radical, and peroxynitrite) trigger DNA single-strand breakage and PARP activation (97, 100). Peroxynitrite is considered a key trigger of DNA strand breakage because (as opposed to hydroxyl radical, for instance) it can travel significant distances and readily crosses cell membranes. When activated by DNA single-strand breaks, PARP initiates an energy-consuming cycle by transferring ADP-ribose units from NAD<sup>+</sup> to nuclear proteins. This process results in rapid depletion of the intracellular NAD<sup>+</sup> and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and cell death (for review, see 114). It is noteworthy that, in addition to the process of NAD<sup>+</sup> depletion and the induction of cellular dysfunction, part of the PARP overactivation-induced cell dysfunction and necrosis is related to intracellular acidification. Part of this process is related to inhibition of sodium/hydrogen exchange in energy-depleted cells (37). Another part of this process is due to a direct acidification: when PARP catabolizes NAD<sup>+</sup>, in addition to ADP-ribose and nicotinamide a "by-product" of the reaction is H<sup>+</sup>, which directly induces intracellular acidification, with direct consequences for cell viability (1).

The PARP-mediated pathway of cell necrosis and the PARP-mediated pathway of inflammatory signal transduction and gene expression may be interrelated in pathophysiological conditions. Oxidant stress can generate DNA single-strand breaks. DNA strand breaks then activate PARP, which in turn potentiates NF- $\kappa$ B activation and AP-1 expression, resulting

in greater expression of the AP-1- and NF- $\kappa$ B-dependent genes, such as the gene for ICAM-1, as well as chemokines such as macrophage inflammatory protein-1 $\alpha$  and cytokines such as tumor necrosis factor- $\alpha$ . Chemokine generation, in combination with increased endothelial expression of ICAM-1, recruits more activated leukocytes to inflammatory foci, producing greater oxidant stress. It is possible that a low-level, localized inflammatory response may be beneficial in recruiting mononuclear cells to an inflammatory site. However, in many pathophysiological states, the above-described feedback cycles amplify themselves beyond control.

### ROLE OF PARP ACTIVATION IN THE PATHOGENESIS OF ENDOTHELIAL DYSFUNCTION

The contribution of the PARP pathway to the development of endothelial dysfunction was proposed in 1997 (98), using an endotoxic shock model in the rat. This model is known to induce severe oxidative and nitrosative stress in the vicinity of the vascular endothelium, due to the up-regulation of iNOS, as well as the activation of various superoxide-generating sources, including NADPH oxidase. In vascular rings taken from rats subjected to endotoxic shock, there was a loss of the endothelium-dependent relaxations, and these alterations were prevented by pharmacological inhibition of PARP with 3-aminobenzamide (98). In *in vitro* studies, vascular rings exposed to peroxynitrite also exhibited reduced endothelium-dependent relaxations in response to acetylcholine, and the development of this endothelial dysfunction was ameliorated by 3-aminobenzamide (98). These findings were consistent with previous *in vitro* data demonstrating that PARP inhibition protects against the metabolic suppression and death of oxidatively (3, 41, 44, 46, 108) or nitrosatively (98) injured endothelial cells. These findings were also consistent with studies where endothelial cells were incubated *in vitro* with various pathophysiological relevant factors that induce oxidative stress, including homocysteine (a model of a variety of cardiovascular diseases) (7) or elevated glucose concentrations (a model of diabetic vascular complications) (33).

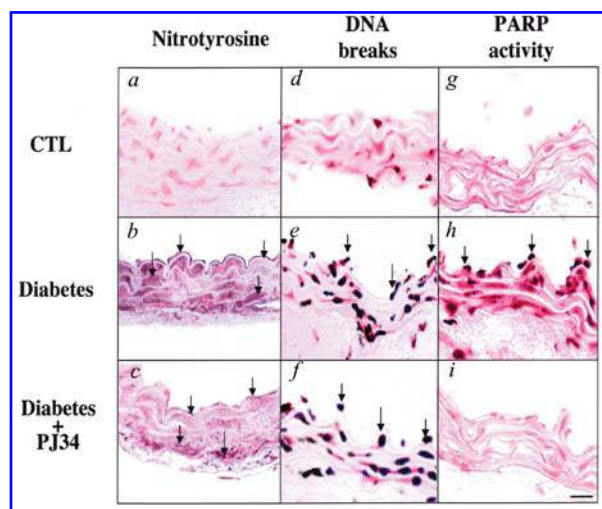
Over the last 5 years, the list of pathophysiological conditions where the endothelial dysfunction has been demonstrated to be dependent on PARP activation has increased. The list, in addition to various forms of shock (20, 42, 52, 69, 98), now includes complement-mediated endothelial injury (22), myocardial infarction and various forms of myocardial reperfusion injury, and heart transplantation (106, 120), as well as the endothelial dysfunction associated with chronic heart failure (70, 71), aging (73), hypertension (74), and diabetes mellitus (33, 72, 91, 92, 102).

### ENDOTHELIAL DYSFUNCTION IN EXPERIMENTAL MODELS OF DIABETES: THE ROLE OF PARP ACTIVATION

Endothelial dysfunction has been documented in various forms of diabetes, and even in prediabetic individuals (11, 12, 14, 19, 86, 102). The pathogenesis of this endothelial dys-

function involves many components, including increased polyol pathway flux, altered cellular redox state, increased formation of diacylglycerol and the subsequent activation of specific protein kinase C (PKC) isoforms, and accelerated nonenzymatic formation of advanced glycation end products (AGEs) (5, 8, 15, 25, 34, 36, 58). Many of these pathways, in concert, trigger the production of oxygen- and nitrogen-derived oxidants and free radicals, such as superoxide anion and peroxynitrite, which play a significant role in the pathogenesis of the diabetes-associated endothelial dysfunction and other diabetic complications. The cellular sources of reactive oxygen species such as superoxide anion are multiple and include AGEs, NADH/NADPH oxidases, the mitochondrial respiratory chain, xanthine oxidase, the arachidonic acid cascade (lipoxygenase and cyclooxygenase), and microsomal enzymes (8, 15, 25, 34).

In a recent study, we have shown that high glucose-induced oxidative and nitrosative stress leads to DNA single-strand breakage and PARP activation in murine and human endothelial cells (33) (Fig. 1). The involvement of oxyradicals and nitric oxide (NO)-derived reactive species in PARP activation and the evidence for nitrated tyrosine residues both suggested that peroxynitrite may be one of the final mediators responsible for single-strand breakage and subsequent PARP activation (33). The role of hyperglycemia-induced oxidative stress in producing DNA damage is also supported by recent findings showing that increased amounts of 8-hydroxyguanine and 8-hydroxydeoxyguanosine (markers of oxidative damage



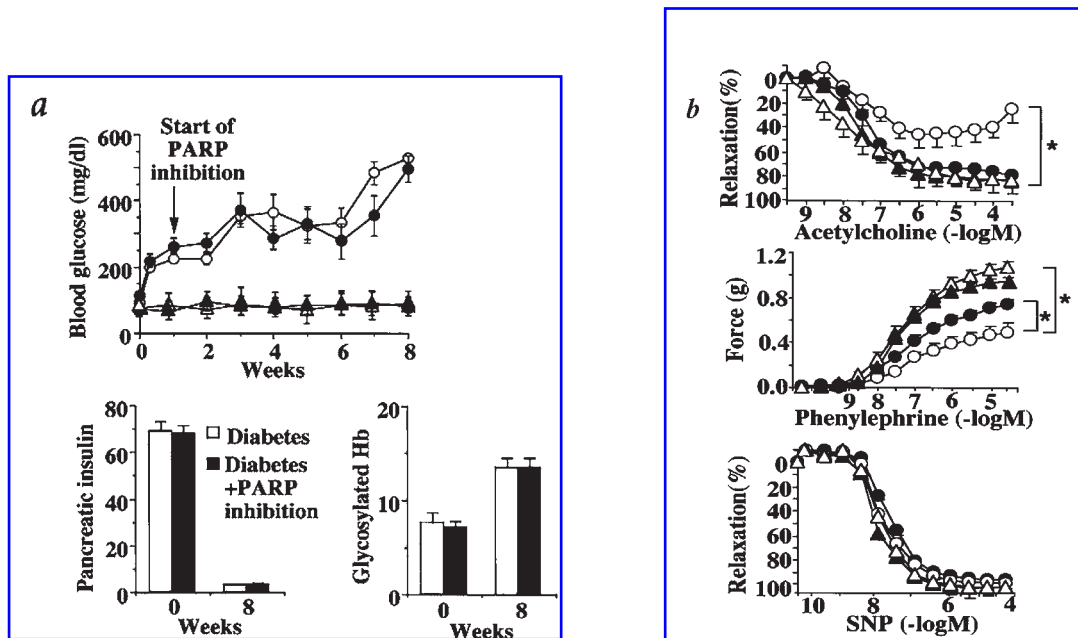
**FIG. 1. Reactive nitrogen species generation, DNA breakage, and PARP activation in diabetic blood vessels.** (a–c) Immunohistochemical staining for nitrotyrosine in control rings (a), in rings from diabetic mice treated with vehicle at 8 weeks (b), and in rings from diabetic mice treated with PJ34 (c). (d–f) Terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labeling, an indicator of DNA-strand breakage, in control rings (d), in rings from diabetic mice treated with vehicle at 8 weeks (e), and in rings from diabetic mice treated with PJ34 (f). (g–i) Immunohistochemical staining for poly(ADP-ribose), an indicator of PARP activation, in control rings (g), in rings from diabetic mice treated with vehicle at 8 weeks (h), and in rings from diabetic mice treated with PJ34 (i). Reproduced with permission from 33.

to DNA) can be found in both the plasma and tissues of streptozotocin (STZ) diabetic rats (78). Importantly, various forms of oxidant-induced DNA damage (base modifications as well as DNA strand breaks) have also been demonstrated in diabetic patients (2, 4, 26, 53, 87).

In a STZ-induced murine model of type I diabetes, we observed that the diabetes-associated loss of endothelial function is not only preventable, but also rapidly reversible with PARP inhibition (33, 91). Intravascular PARP activation (seen primarily in endothelial cells, as well as in vascular smooth muscle cells) was already apparent 2 weeks after the onset of diabetes, and thus it slightly preceded the occurrence of the endothelial dysfunction, which developed between the 2<sup>nd</sup> and the 4<sup>th</sup> week of diabetes (33, 91) (Fig. 2). Delayed treatment with the PARP inhibitor, starting at 1 week after STZ, ameliorated vascular poly(ADP-ribose) accumulation and restored normal vascular function without altering systemic glucose levels, plasma glycated hemoglobin levels, or pancreatic insulin content (33, 91). Furthermore, delayed treatment of the animals with the PARP inhibitor restored the already established diabetic endothelial dysfunction (Fig. 3), and even *in vitro* incubation of diabetic blood vessels with PARP inhibitors of various structural classes significantly enhanced their endothelium-dependent relaxant responsiveness (91) (Fig. 4). The development of the endothelial dysfunction and its reversibility by pharmacological inhibition of PARP have also been demonstrated in an autoimmune model of diabetes (72) (Fig. 5). The endothelial dysfunction was associ-

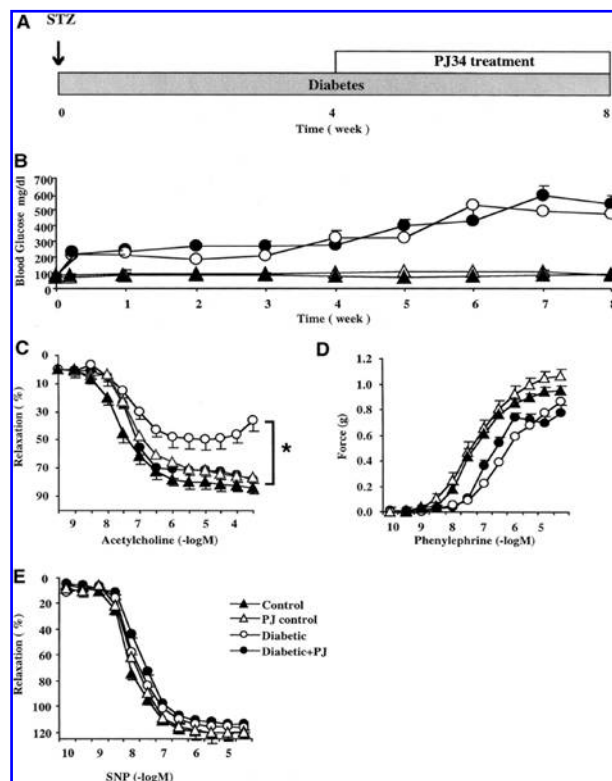
ated with a simultaneous loss of NAD<sup>+</sup> and NADPH in the vasculature, and PARP inhibition reversed these changes. Based on these observations, and the known fact that endothelial nitric oxide synthase (eNOS; the NOS isoform present in the vascular endothelial cells) is dependent on NADPH and is sensitively regulated by this cofactor, we hypothesized that the endothelial dysfunction in diabetes is dependent on a PARP-mediated, reversible cellular NADPH deficiency (33, 91). In fact, previous *in vitro* work demonstrated that the NADPH depletion in oxidatively stressed cells is dependent on PARP activation (18, 39, 47). It is interesting to note that other groups have demonstrated that diabetic endothelial dysfunction is also associated with direct oxidation and consequent cellular depletion of other cofactors of eNOS, such as tetrahydrobiopterin (32, 33, 35, 75, 117). As in the absence of tetrahydrobiopterin a functional uncoupling of eNOS occurs and the enzyme produces superoxide and peroxynitrite, rather than NO (114), the consequences of these processes are increased free radical and oxidant production, oxidative damage, and further exacerbation of the endothelial dysfunction.

The mode of the protective action of PARP inhibitors on the vascular endothelium *in vivo* likely involves the conservation of cellular energetic pools, as well as a prevention of the up-regulation of various proinflammatory pathways [cytokines, adhesion molecules (ICAM-1, VCAM-1, and E-selectin) mononuclear cell infiltration] triggered by hyperglycemia (16, 33, 92). This latter mechanism may represent an important additional pathway whereby PARP activation



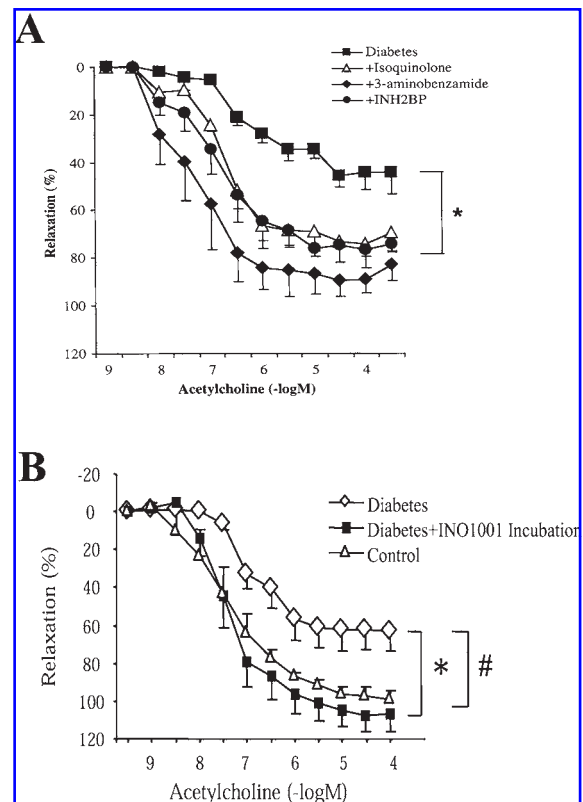
**FIG. 2. Reversal of diabetes-induced endothelial dysfunction by pharmacological inhibition of PARP.** The following symbols were used for the respective groups: animals that received no STZ injection ( $\Delta$ ), nondiabetic control animals at 8 weeks treated with PJ34 between week 1 and 8 ( $\blacktriangle$ ), diabetic animals at 8 weeks treated with vehicle ( $\circ$ ), diabetic animals at 8 weeks treated with PJ34 between week 1 and 8 ( $\bullet$ ). (a) Blood glucose levels, pancreatic insulin content (ng of insulin/mg of pancreatic protein), and blood glycosylated hemoglobin (Hb) (expressed as % of total Hb) at 0–8 weeks in nondiabetic, control male BALB/c mice, and at 0–8 weeks after STZ treatment (diabetic) in male BALB/c mice. PARP inhibitor treatment, starting at 1 week after STZ and continuing until the end of week 8, is indicated by the arrow. Pancreatic insulin and glycosylated hemoglobin levels are shown at 8 weeks in vehicle-treated and STZ-treated animals, in the presence or absence of PJ34 treatment. (b) Acetylcholine-induced, endothelium-dependent relaxations, phenylephrine-induced contractions, and sodium nitroprusside (SNP)-induced endothelium-independent relaxations. \* $p < 0.05$  for vehicle-treated diabetic versus PJ34-treated diabetic mice ( $n = 8$  per group). Reproduced with permission from 33.





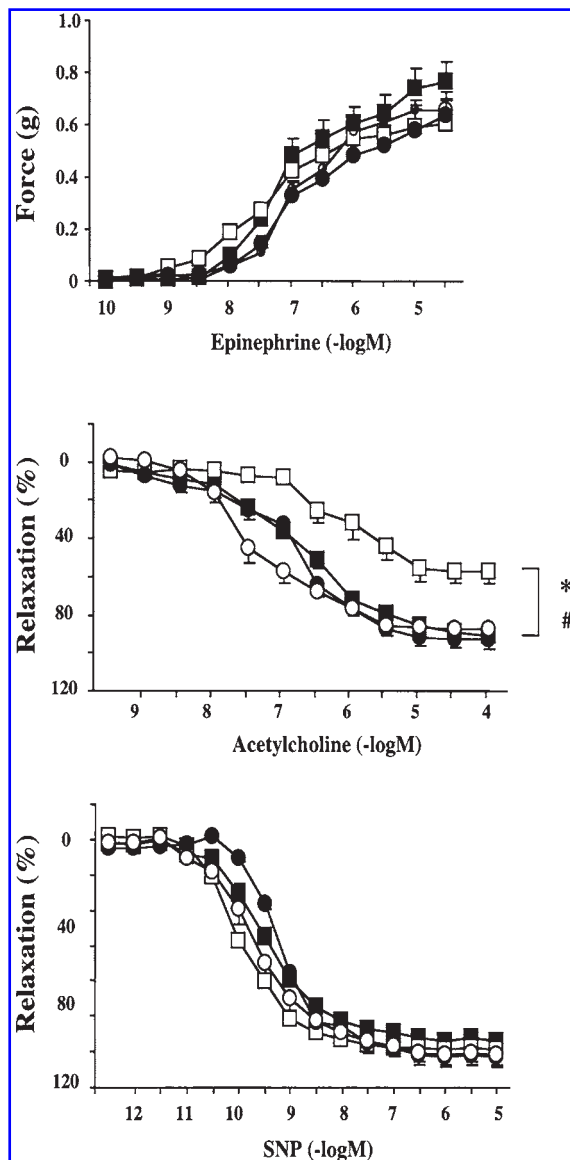
**FIG. 3. Pharmacological inhibition of PARP restores impaired endothelium-dependent relaxant ability of the diabetic vessels.** Blood glucose levels and vascular responsiveness are presented. Endothelium-dependent relaxations were induced by acetylcholine, contractions induced by phenylephrine, and endothelium-independent relaxations induced by sodium nitroprusside (SNP) in control (nondiabetic) male Balb/c mice and 1, 4, and 8 weeks after STZ-induced diabetes. Vehicle or PARP inhibitor (PJ34, 10 mg/kg oral gavage once a day) treatment started at 4 weeks after STZ and continued until 8 weeks (the end of the experimental period). There was a marked and selective impairment of the endothelium-dependent relaxant ability of the vascular rings in diabetes at 4 and 8 weeks. Treatment with the PARP inhibitor between weeks 4 and 8 restored to normal the endothelium-dependent relaxant ability of the diabetic vessels despite the persistence of hyperglycemia. \* $p < 0.05$  for differences between experimental groups, as indicated.  $n = 8$  per group. Reproduced with permission from 91.

can contribute to vascular dysfunction via the up-regulation of adhesion molecules. As mentioned earlier, PARP regulates the activation of a variety of signal transduction pathways, and some of these pathways regulate the expression of cell surface and soluble adhesion molecules. Recent preliminary data indicate that pharmacological inhibition of PARP can suppress this process (16). Intermittent high/low glucose induces a more pronounced expression of adhesion molecules than constant high glucose (82), and PARP inhibition suppresses NF- $\kappa$ B activation and the expression of adhesion molecules both under constant high glucose and under intermittent high/low glucose conditions in cultured endothelial cells *in vitro* (16).



**FIG. 4. *In vitro* treatment with all PARP inhibitors improved the endothelium-dependent relaxant ability of the diabetic vessels.** (A) Endothelium-dependent relaxations induced by acetylcholine in control (nondiabetic) male Balb/c mice and 4 weeks after STZ-induced diabetes. In a subgroup of the vascular rings, evaluation of vascular responsiveness was preceded by 1-h incubation with three structurally different PARP inhibitors: 3-aminobenzamide (3 mmol/L), 5-iodo-6-amino-1,2-benzopyrone (INH2BP) (100  $\mu$ mol/L), or 1,5-dihydroxyisoquinoline (Isoquinolone) (30  $\mu$ mol/L). There was a marked and selective impairment of the endothelium-dependent relaxant ability of the vascular rings in diabetes at 4 weeks. *In vitro* treatment with all PARP inhibitors improved the endothelium-dependent relaxant ability of the diabetic vessels. \* $p < 0.05$  for differences between experimental groups, as indicated.  $n = 8$  per group. Reproduced with permission from 91. (B) Endothelium-dependent relaxations induced by acetylcholine in control (nondiabetic) male Balb/c mice and 6 weeks after STZ-induced diabetes. In a subgroup of the vascular rings, evaluation of vascular responsiveness was preceded by 1-h incubation with the novel potent PARP inhibitor, INO1001 (3  $\mu$ mol/L). There was a marked and selective impairment of the endothelium-dependent relaxant ability of the vascular rings in diabetes at 6 weeks. *In vitro* treatment with all PARP inhibitors improved the endothelium-dependent relaxant ability of the diabetic vessels. #, \* $p < 0.05$  for differences between experimental groups, as indicated.  $n = 8$  per group.

Brownlee and colleagues have demonstrated that the hyperglycemia-induced overproduction of superoxide by mitochondrial electron-transport chain activated major pathways of hyperglycemic damage found in aortic endothelial cells



**FIG. 5. Reversal of diabetes-induced endothelial dysfunction by pharmacological inhibition of PARP in diabetic NOD mouse vascular rings.** Epinephrine-induced contractions (**upper panel**), acetylcholine-induced endothelium-dependent relaxation (**middle panel**), and sodium nitroprusside (SNP)-induced endothelium-independent relaxations (**lower panel**). ■, control; ○, control + PJ34; □, diabetes; ●, diabetes + PJ34. Each point of the curve represents the mean  $\pm$  SE of five to eight experiments in vascular rings. \* $p < 0.05$  versus control; # $p < 0.05$  versus diabetes. Reproduced with permission from 72.

(activation of PKC isoforms, hexosamine pathway flux, and AGE formation) by inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity (28, 29, 83). Importantly, the hyperglycemia-induced GAPDH inhibition was found to be a consequence of poly(ADP-ribosyl)ation of GAPDH by PARP, which was activated by DNA strand breaks produced by reactive species generated by hyperglycemia. One of the likely DNA-damaging factors is peroxynitrite, which is gen-

erated when mitochondrial superoxide reacts with NO produced by the constitutive eNOS. Both the hyperglycemia-induced decrease in activation of GAPDH and its poly (ADP-ribosyl)ation can be prevented by overexpression of either uncoupling protein-1 (UCP-1) or manganese superoxide dismutase (MnSOD), which decrease hyperglycemia-induced superoxide generation. Overexpression of UCP-1 or MnSOD also prevented hyperglycemia-induced DNA strand breaks and activation of PARP (28). Similarly, administration of the mitochondrial uncoupler 2,4-dinitrophenol to endothelial cells exposed to high glucose blocked glucose-induced DNA strand breakage and PARP activation (76). Importantly, the hyperglycemia-induced activation of PKC isoforms, hexosaminase pathway flux, and AGE formation was prevented by blocking PARP activity with various structurally unrelated inhibitors of the enzyme (28).

An additional factor to be considered in the context of PARP activation and the pathogenesis of endothelial dysfunction and diabetic complications is angiotensin II. Angiotensin II is a known factor in the pathogenesis of diabetic complications, perhaps most importantly in nephropathy, cardiomyopathy, and retinopathy. Recent studies indicate that the protective effects of angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists may go beyond the blood pressure-lowering effects of these agents (6, 9, 50). In this context, it is noteworthy that angiotensin II can induce direct, prooxidative effects on the vascular endothelium. These effects are, at least in part, mediated by intraendothelial reactive species formation via a new family of NAD(P)H oxidase subunits, known as the nonphagocytic NAD(P)H oxidase proteins. Reactive oxidant species produced following angiotensin II-mediated stimulation of NAD(P)H oxidases can exert direct oxidative effects, but can also signal through pathways such as mitogen-activated protein kinases, tyrosine kinases, and transcription factors, and lead to events such as inflammation, hypertrophy, remodeling, and angiogenesis (13). Recent work demonstrates that angiotensin II can also induce intraendothelial peroxynitrite formation (56, 116), as well as PARP activation (104). Administration of angiotensin II triggers the activation of PARP in cultured endothelial cells *in vitro*. The *in vitro* PARP activation is dose-dependently inhibited by PARP inhibitors of various structural classes, as well as by the compound apocynin, indicating that NAD(P)H oxidase-generated superoxide anion accounts for the generation of the reactive species that trigger DNA single strand breakage and PARP activation (104). Angiotensin-induced PARP activation is also inhibited by *N* $^{\omega}$ -nitro-L-arginine methyl ester and diphenyleneiodonium (104). Thus, angiotensin triggers the endothelial generation of reactive oxygen species from NAD(P)H oxidase, and these with constitutively produced NO produce peroxynitrite and other reactive nitrogen species, which induce DNA breakage and activate PARP in the vascular endothelium, leading to the development of endothelial dysfunction. This pathway is also operative *in vivo*, as chronic infusion of subpressor doses of angiotensin infusion triggers endothelial dysfunction *in vivo*, which can be prevented or reversed by PARP inhibition (103). Future work needs to establish the importance of this pathway in the context of diabetic complications.

## ENDOTHELIAL DYSFUNCTION IN DIABETIC AND PREDIABETIC PATIENTS: THE POTENTIAL ROLE OF PARP ACTIVATION

A recent study of forearm skin biopsies from healthy subjects extended our knowledge on the role of PARP activation in the development of diabetic endothelial dysfunction in human subjects. Analysis of dermal biopsy samples from healthy individuals with parental history of type 2 diabetes (T2DM), subjects with impaired glucose tolerance, and a group of type 2 diabetic patients indicated that the percentage of PARP-positive endothelial nuclei was higher in the group of parental history of T2DM and diabetic patients when compared with the controls (102). In addition, significant correlations were observed between the percentage of PARP-positive endothelial nuclei and fasting blood glucose, resting skin blood flow, maximal skin vasodilatory response to the iontophoresis of acetylcholine (which indicates endothelium-dependent vasodilation), and nitrotyrosine immunostaining intensity. Nitrotyrosine immunoreactivity [a marker of reactive nitrogen species (chiefly peroxynitrite) formation] was also higher in the diabetic patients when compared with all other groups (102). Significant correlations were observed between nitrotyrosine immunostaining intensity and fasting blood glucose, glycosylated hemoglobin (HbA1c), ICAM, and VCAM. No differences in the expression of eNOS and RAGE were found among all four groups. The polymorphism of the eNOS gene was also studied and was not found to influence eNOS expression or microvascular functional measurements. Thus, in humans, PARP activation is present in healthy subjects at risk of developing diabetes, as well as in established type 2 diabetic patients, and it correlates with impairments in the vascular reactivity in the skin microcirculation (102). As interventional studies with PARP inhibitors in humans with diabetic endothelial dysfunction have not yet been conducted, it remains to be seen whether PARP activation in diabetic or prediabetic humans can be seen as a predictor or early marker for the development of diabetic vascular complications.

### THE ROLE OF PARP ACTIVATION IN THE PATHOGENESIS OF DIABETIC CARDIOMYOPATHY

It is well established that the superoxide–peroxynitrite–PARP pathway plays a pivotal role in various models of myocardial ischemia–reperfusion injury (a condition in which oxidative and nitrosative stress plays a key pathogenetic role) (106, 107, 120). Recent data demonstrate that the PARP pathway also plays a pathogenetic role in the development of diabetic cardiomyopathy (72). Cardiac dysfunction and PARP activation in the cardiac myocytes and the coronary vasculature were observed in both STZ-induced and genetic (nonobese diabetic) models of diabetes mellitus in rats and mice. Furthermore, treatment with the phenanthridinone-based PARP inhibitor PJ34, starting 1 week after the onset of diabetes,

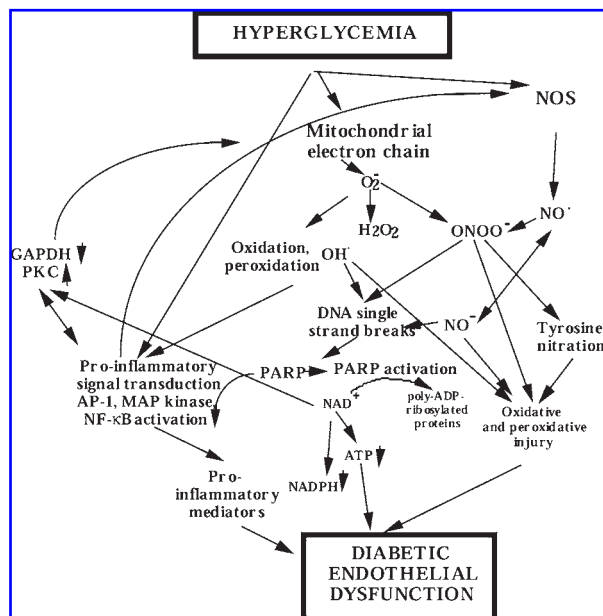
restored normal vascular responsiveness and significantly improved cardiac function in diabetic mice and rats, despite the persistence of severe hyperglycemia. The beneficial effect of PARP inhibition persisted even after several weeks of the discontinuation of the PARP inhibitor treatment (72).

It is conceivable that the diabetic endothelial PARP pathway and the diabetic cardiomyopathy are interrelated: the impairment of the endothelial function may lead to global or regional myocardial ischemia, which may secondarily impair cardiac performance. The beneficial effect of PARP inhibition on myocardial function, however, is not related to an anabolic effect because PJ34 treatment did not influence the body and heart weight loss in diabetic animals, whereas it dramatically improved cardiac function. It is noteworthy that the protective effect of PARP inhibition against diabetic cardiac dysfunction extends several weeks beyond the discontinuation of treatment; this observation may have important implications for the design of future clinical trials with PARP inhibitors. The prolonged protective effect may be related to the permanent interruption by the PARP inhibitor of positive feedback cycles of cardiac injury. Indeed, previous studies in various pathophysiological conditions have demonstrated that PARP inhibitors suppress positive feedback cycles of adhesion receptor expression and mononuclear cell infiltration, as well as cellular oxidant generation (16, 106, 120). The mode of the PARP inhibitors' cardioprotective action involves a conservation of myocardial energetics, as well as a prevention of the up-regulation of various proinflammatory pathways (cytokines, adhesion receptors, mononuclear cell infiltration) triggered by ischemia and reperfusion (106, 120). It is conceivable that PARP inhibition exerts beneficial effects in experimental models of diabetic cardiomyopathy by affecting both above-referenced pathways of injury, and also by suppressing positive feedback cycles initiated by them.

### THE ROLE OF PARP ACTIVATION IN THE PATHOGENESIS OF DIABETIC RETINOPATHY, NEPHROPATHY, AND NEUROPATHY

Although most of the studies on the role of PARP in the pathogenesis of diabetic endothelial dysfunction were conducted in macrovessels (see above), there is circumstantial evidence that similar processes are operative for the pathogenesis of diabetic microvascular injury (which is an important underlying mechanism for the pathogenesis of retinopathy, nephropathy, and neuropathy). In fact, there is now evidence of PARP activation in the microvessels and ganglionic layer of the diabetic retina (64, 105). The causative role of PARP in diabetic retinopathy is now supported by two independent interventional preclinical studies. In one report (119), a long-term (9-month) study was used to investigate the role of PARP in hyperglycemia-induced cell death *in vitro* and in the development of diabetic retinopathy *in vivo*. STZ-diabetic Lewis rats were treated with vehicle or the PARP inhibitor PJ34. Diabetes was found to increase activity of PARP in retina measured at 2 months, and PJ34 inhibited this increase. PARP activation was detectable also in a subset of nu-

clei from retinal capillary endothelial cells and pericytes. Diabetes of 9 months duration significantly increased the number of both TUNEL-positive capillary cells and acellular capillaries (a marker of degenerate capillaries), and PJ34 significantly inhibited these alterations without influencing glycemic control. PJ34 also inhibited a diabetes-induced up-regulation of ICAM and leukostasis within the retinal vascu-



**FIG. 6. Overview of the role of PARP in regulating multiple components of hyperglycemia-induced endothelial dysfunction.** High circulating glucose interacts with the vascular endothelium where it triggers the release of oxidant mediators from the mitochondrial electron transport chain, as well as from NADH/NADPH oxidase and other sources. NO, in turn, combines with superoxide ( $O_2^-$ ) to yield peroxynitrite ( $ONOO^-$ ). Hydroxyl radical ( $OH^\cdot$ ) (produced from superoxide via the iron-catalyzed Haber-Weiss reaction) and peroxynitrite or peroxynitrous acid induce the development of DNA single-strand breakage, with consequent activation of PARP. Depletion of the cellular  $NAD^+$  leads to inhibition of cellular ATP-generating pathways leading to cellular dysfunction. The PARP-triggered depletion of cellular NADPH directly impairs the endothelium-dependent relaxations. The effects of elevated glucose are also exacerbated by increased aldose reductase activity leading to depletion of NADPH and generation of reactive oxidants. NO alone does not induce DNA single-strand breakage, but may combine with superoxide (produced from the mitochondrial chain or from other cellular sources) to yield peroxynitrite. Under conditions of low cellular L-arginine, NOS may produce both superoxide and NO, which then can combine to form peroxynitrite. PARP activation, via a not yet characterized fashion, can promote the activation of nuclear factor- $\kappa$ B, AP-1, mitogen-activated protein (MAP) kinases, and the expression of proinflammatory mediators, adhesion molecules, and iNOS. PARP activation contributes to the activation of PKC. PARP activation also leads to the inhibition of cellular GAPDH activity, at least in part via the direct poly(ADP-ribosylation) of GAPDH. PARP-independent, parallel pathways of cellular metabolic inhibition can be activated by NO, hydroxyl radical, superoxide, and peroxynitrite.

lature. In a complementary *in vitro* study, bovine retinal endothelial cells and pericytes were incubated in 5 mM (normal) and 25 mM (elevated) glucose for 5 days with or without PJ34. High glucose significantly increased death of retinal capillary endothelial cells, and PARP inhibition prevented this cell death. In a second, independent study (119), male C57/BL6 mice were rendered diabetic with a single injection of STZ. Diabetic mice, treated with the PARP inhibitor PJ34 for 6 months, were investigated for experimental retinopathy by using retinal digest preparations and quantitative retinal morphometry. Diabetes over 6 months induced pericyte loss and increased the number of acellular capillaries. Treatment with PJ34 inhibited both the loss of pericytes and the formation of acellular capillaries. These data, taken together, suggest that hyperglycemia-induced PARP activation affects predominantly the retinal vasculature and is susceptible to pharmacological PARP inhibition.

As far as the role of PARP in diabetic nephropathy goes, the presence of glomerular depositions (mesangial distribution) of IgG was significantly reduced in STZ-diabetic rats treated with the PARP inhibitor nicotinamide for 6 months (115). In agreement with these results, we have recently provided evidence that PARP activation is present in the tubuli of STZ-induced diabetic rats. This PARP activation is attenuated by two unrelated PARP inhibitors, 3-aminobenzamide and 1,5-isoquinolinediol, which also counteracted the overexpression of endothelin-1 and endothelin receptors in the renal cortex (57).

It has recently been suggested that the oxidative/nitrosative stress-PARP pathway PARP also plays a key role in the development of diabetic neuropathy: the progressive slowing of sensory and motor neuron conductance in diabetic rats and mice is preventable by PARP inhibition or PARP deficiency, and this is associated with maintained neuronal phosphocreatine levels, as well as improved endoneurial blood flow (17, 51, 61, 62, 76). Importantly, pharmacological PARP inhibition is not only a preventive option; it can also restore sensory and motor neuronal conduction in already established diabetic neuropathy, at least in murine models of the disease (51).

Additional studies, utilizing potent and specific inhibitors of PARP, are needed to further delineate the role of PARP in the pathogenesis of diabetic retinopathy, neuropathy, and nephropathy. It is important to reemphasize that, although the above conditions are generally considered as separate pathophysiological entities, there is good evidence that, at least in part, they all develop on the basis of endothelial (vascular) dysfunction (59, 60). As diabetic erectile dysfunction is also known to develop on the basis of diabetic endothelial dysfunction and diabetic neuropathy (84), the potential role of PARP activation in this condition must also be explored in future studies.

## CONCLUSIONS AND IMPLICATIONS

Based on the evidence reviewed herein, we conclude that the PARP pathway plays very important regulatory roles in the pathogenesis of vascular endothelial dysfunction in pathophysiological conditions associated with oxidative stress, including diabetes (20, 75, 76, 110). It remains to be studied



whether various clinical therapeutic or experimental therapeutic interventions, which are known to have some vascular protective effects in diabetes (antioxidant therapies, peroxisome proliferator-activated receptor agonists, etc.), are able to suppress the activation of PARP in the cardiovascular system. It is noteworthy in this respect that, in preclinical studies, administration of the aldose reductase inhibitors sorbinil or fidarestat to diabetic rats not only corrected diabetes-induced depletion of glutathione and ascorbate, down-regulation of superoxide dismutase activity, and accumulation of lipid peroxidation products in the peripheral nerve, counteracted superoxide formation in *vasa nervorum*, and was effective against multiple indices of diabetes-associated retinal oxidative and nitrosative stress, but also inhibited poly(ADP-ribose) accumulation (a marker of PARP activation) in diabetic nerve and retina (65). Similar results were obtained with FP15, a novel peroxynitrite decomposition catalyst compound (63, 76, 101). In a murine study, sciatic motor nerve conduction velocity and hind-limb digital sensory conduction velocity were reduced in diabetic mice versus controls, and both indices were normalized by FP15, which also ameliorated the accumulation of poly(ADP-ribose) accumulation in diabetic nerves (63).

The pathogenetic role of the oxidative/nitrosative stress–PARP pathway (Fig. 6) is not limited to the diabetes-induced vascular dysfunction, but it has also been demonstrated in various animal models of other diabetic complications, including cardiomyopathy, nephropathy, neuropathy, and retinopathy. PARP activation, thus, is a unique checkpoint in the development and progression of various diabetic complications. PARP inhibition may emerge as a novel approach for the prevention or reversal of diabetic complications. The benefits and potential risks associated with chronic administration of PARP inhibitors are discussed in a recent review (94). The comparative therapeutic utility of PARP inhibition for the experimental therapy of diabetic complications should be explored by additional preclinical and subsequent clinical investigations.

## ABBREVIATIONS

AGE, advanced glycation end product; AP-1, activator protein-1; eNOS, endothelial nitric oxide synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible nitric oxide synthase; MnSOD, manganese superoxide dismutase; NAD<sup>+</sup>, nicotinamide adenine dinucleotide; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NO, nitric oxide; PARG, poly(ADP-ribose) glycohydrolase; PARP/PARS, poly(ADP-ribose) polymerase/synthase; PJ34, potent water-soluble phenanthridinone-derived PARP inhibitor; PKC, protein kinase C; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; UCP-1, uncoupling protein-1; VCAM-1, vascular cellular adhesion molecule-1.

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