The Role of Poly(ADP-Ribose) Polymerase Activation in the Development of Myocardial and Endothelial Dysfunction in Diabetes

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Patients with diabetes exhibit a high incidence of diabetic cardiomyopathy and vascular complications, which underlie the development of retinopathy, nephropathy, and neuropathy and increase the risk of hypertension, stroke, and myocardial infarction. There is emerging evidence that the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) importantly contributes to the development of endothelial dysfunction in a streptozotocin-induced model of diabetes. We investigated the role of PARP activation in the pathogenesis of cardiac dysfunction in streptozotocin-induced and genetic (nonobese diabetic) models of diabetes in rats and mice. Development of diabetes was accompanied by hyperglycemia, cardiac PARP activation, a selective loss of endothelium-dependent vasodilation in the thoracic aorta, and an early diastolic dysfunction of the heart. Treatment with a novel potent phenanthridinone-based PARP inhibitor, PJ34, starting 1 week after the onset of diabetes, restored normal vascular responsiveness and significantly improved cardiac dysfunction, despite the persistence of severe hyperglycemia. The beneficial effect of PARP inhibition persisted even after several weeks of discontinuation of the treatment. Thus, PARP activation plays a central role in the pathogenesis of diabetic cardiovascular (cardiac as well as endothelial) dysfunction. PARP inhibitors may exert beneficial effects against the development of cardiovascular complications in diabetes. *Diabetes* 51:514–521, 2002

Poly(ADP ribose) polymerase (PARP), also known as poly(ADP ribose) synthetase (PARS), is an abundant nuclear enzyme of eukaryotic cells that participates in DNA repair in response to genotoxic stress. When activated by DNA single-strand breaks, PARP initiates an energy-consuming cycle by transferring ADP ribose units from NAD^+ to nuclear proteins. This process results in rapid depletion of the intracellular NAD^+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and death (1–7). Overactivation of PARP represents an important mechanism of tissue damage in various pathological conditions associated with oxidant stress, including myocardial reperfusion injury (4), stroke (3), shock (7,8), and autoimmune β-cell destruction (5,6). Recently, we reported that the activation of PARP importantly contributes to the development of endothelial dysfunction in a streptozotocin (STZ)-induced model of diabetes in mice (9,10).

Cardiovascular complications are the most common cause of morbidity and mortality in diabetic patients. The presence of myocardial dysfunction independent of coronary artery disease in diabetes, known as "diabetic cardiomyopathy," has been well documented in both humans and animals (11–15). Diabetic cardiomyopathy is characterized by an early diastolic dysfunction and a late systolic one, with intracellular retention of calcium and sodium and loss of potassium. The mechanism of diastolic dysfunction remains unknown, but it does not appear to be due to changes in blood pressure, microvascular complications, or elevated circulating glycated hemoglobin levels (15,16). We tested whether the impairment of cardiac function in diabetes is dependent upon the activation of the PARP pathway within the heart.

RESEARCH DESIGN AND METHODS

The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by U.S. National Institutes of Health (NIH publication no. 85-23, revised 1985) and was performed with the approval of the local Institutional Animal Care and Use Committee.

Experimental models of diabetes

Genetic model of diabetes in mice. A total of 72 adult female NOD mice weighing (20–30 g) were used for studies. Mice developing severe hyperglycemia (>350 mg/dl) and glucosuria for two consecutive days were selected and considered diabetic (n = 40). NOD mice with normal (<200 mg/dl) blood and urine glucose concentrations were used as controls (n = 32). PARP was inhibited by PJ34 (10 mg/kg oral gavage, once daily, starting 1 week after the development of diabetes). This dose regimen was found to effectively inhibit vascular PARP activation in previous studies (9,10). Diabetic and control mice were killed after 4 weeks of treatment with PJ34 or vehicle.

STZ-induced model of diabetes in rats. Diabetes was induced in male Wistar rats (n = 50) weighing 240–260 g by use of a single injection of STZ (80 mg/kg i.v.) dissolved in the citrate-saline solution (pH 4.5) into the tail vein. Control animals (n = 36) were injected with the vehicle alone. Diabetes was confirmed by the presence of hyperglycemia (>200 mg/dl). One week after the injection of STZ, control and diabetic rats received either vehicle or the PARP

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ABC, avidin-biotin-peroxidase complex; DAB, diaminobenzidine; +dP/dt, systolic pressure increment; −dP/dt, systolic pressure decrement; PARP, poly(ADP-ribose) polymerase; STZ, streptozotocin.

inhibitor PJ34 (10 mg/kg oral gavage, once daily). Rats and age-matched controls were killed after 4 weeks of treatment.

In a separate set of experiments, diabetic ($n = 12$) and control rats ($n = 12$) were treated with PJ34 (10 mg/kg oral gavage, once daily) for 6 weeks, and then treatment was stopped and animals were followed for a subsequent period of 3 weeks. At 10 weeks, animals were subjected to cardiovascular measurements.

Blood glucose was measured using a one-touch blood glucose meter (Lifescan; Johnson & Johnson, Milpitas, CA). Total glycated hemoglobin was measured using a commercially available kit (Sigma, St. Louis, MO). Pancreatic insulin content in NOD mice was measured in the supernatant of pancreatic homogenates using an enzyme-linked immunosorbent assay as previously described (9).

Measurement of vascular reactivity in isolated aortic rings of NOD mice. The method was described previously in detail (9). Briefly, the thoracic aorta was cleared from periadventitial fat and cut into 0.5–1.0 mm width rings using operation microscope, mounted in organ baths filled with warmed (37°C) and oxygenated (95% O_2/5% CO_2) Krebs' solution ($CaCl_2$ 1.6 mmol/l; $MgSO_4$ 1.17 mmol/l; EDTA 0.026 mmol/l; NaCl 130 mmol/l; $NaHCO_3$ 14.9 mmol/l; KCl 4.7 mmol/l; KH_2PO_4 1.18 mmol/l; and glucose 11 mmol/l). Isometric tension was measured with isometric transducers (Kent Scientific Corporation, Litchfield, CT), digitized using a MacLab A/D converter and stored and displayed on a MacIntosh computer. A tension of 1 g was applied and the rings were equilibrated for 60 min, followed by measurements of the concentration-dependent contraction to epinephrine (10^{-10} to 3×10^{-5} mol/l), and in rings precontracted with epinephrine (10^{-6} mol/l), relaxation to acetylcholine (10^{-9} to 10^{-5} mol/l) and sodium nitroprusside (10^{-12} to 10^{-5} mol/l). Experiments were conducted in 5–8 pairs of rings in each experimental group.

Hemodynamic measurements in mice and rats. Analysis of left ventricular performance was measured in female NOD mice anesthetized with intraperitoneal injections of ketamine (80 mg/kg) and xylazine (10 mg/kg) and in rats anesthetized with intraperitoneal injection of thiopentone sodium (60 mg/kg). The animals were placed on controlled heating pads, and core temperature measured via a rectal probe was maintained at 36–38°C.

A microtip catheter transducer (SPR-671 in mice and SPR-S24 in rats; Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the left ventricle under pressure control. After stabilization for 15–20 min, the pressure signal was continuously recorded using a MacLab A/D converter (AD Instruments, Mountain View, CA) and stored and displayed on an Apple Macintosh personal computer. The heart rate, the left ventricular systolic and end-diastolic pressures were measured, and the maximal slope of systolic pressure increment (+dP/dt) and diastolic pressure decrement (−dP/dt), indexes of contractility and relaxation, were calculated. After these measurements, the catheter was pulled back into the aorta for the measurement of arterial blood pressure. After the hemodynamic measurements were made, animals were killed, and hearts were excised and cardiac weights were recorded followed by fixation of samples in 10% formalin. Slices of the left ventricle were embedded in paraffin for immunohistochemical detection of poly(ADP-ribose) formation.

Immunohistochemical detection of poly(ADP-ribose). Paraffin sections (3 μm of hearts) were deparaffinized in xylene and rehydrated in decreasing concentrations (100, 95, and 75%) of ethanol followed by a 10-min incubation in PBS (pH 7.4). Sections were treated with 0.3% hydrogen peroxide for 15 min to block endogenous peroxidase activity and then rinsed briefly in PBS. Nonspecific binding was blocked by incubating the slides for 1 h in PBS containing 2% horse serum. To detect poly(ADP-ribose), a routine histochemical procedure was applied, as previously described (17) with minor modifications as follows. Mouse monoclonal anti-poly(ADP-ribose) antibody (Alexis, San Diego, CA) and isotype-matched control antibody was applied in a dilution of 1:100 for 2 h at room temperature. After extensive washing (5×5 min) with PBS, immunoreactivity was detected with a biotinylated goat anti–rabbit secondary antibody and the avidin-biotin-peroxidase complex (ABC) both supplied in the Vector Elite kit (Vector Laboratories, Burlingame, CA). Color was developed using Ni-DAB substrate (95 mg diaminobenzidine, 1.6 g NaCl, 2 g nickel sulfate in 200 ml of 0.1 mol/l acetate buffer). Sections were then counterstained with Nuclear Fast Red and were dehydrated and mounted in Permount. Photomicrographs were taken with a Zeiss Axiolab microscope equipped with a Fuji HC-300C digital camera. All histological and immunohistochemical samples were coded and examined and graded by an investigator (E.S.) in a blinded fashion.

In the murine studies, PARP activity in the heart was measured by using an immunohistochemical method of PARP activity utilizing biotinylated NAD (18). Briefly, cryosections (10 μm) were fixed for 10 min in 95% ethanol at −20°C and then rinsed in PBS. Sections were permeabilized by 1% Triton X-100 in 100 mmol/l Tris (pH 8.0) for 15 min. Reaction mixture (10 mmol/l $MgCl_2$, 1 mmol/l dithiothreitol, and 30 μmol/l biotinylated NAD^+ in 100 mmol/l Tris, pH 8.0) was then applied to the sections for 30 min at 37°C. Reaction mixes containing 5 mmol/l 3-aminobenzamide or biotinyl-NAD^+ free reaction mix were used as controls. After three washes in PBS, incorporated biotin was detected by peroxidase-conjugated streptavidine (1:100, 30 min, room temperature). After 3×10 min washes in PBS, color was developed with cobalt-enhanced nickel-DAB substrate. Sections were counterstained in Nuclear Fast Red, dehydrated, and mounted in Vectamount.

Quantification of the intensity of the poly(ADP-ribose) staining was performed as previously published (21,30). The percentage of PARP-positive nuclei of cardiomyocytes was obtained by conventional microscopy; in total, 4,289–5,892 nuclei profiles were examined in each condition, in at least five different animals in each group. The results are expressed as the percent of PARP-positive nuclei of myocytes, relative to the number of total nuclei counted.

Reagents. All reagents were obtained from Sigma/Aldrich (St. Louis, MO), unless indicated otherwise. The potent, novel, water soluble phenanthridinone derivative PARP inhibitor, PJ34—the hydrochloride salt of *N*-(-oxo-5,6-dihydro-phenanthridin-2-yl)-*N*,*N*-dimethylacetamide—was synthesized as described (9). In cell-free PARP assay, using NAD^+ and purified PARP-1 enzyme, PJ34 inhibited PARP activity in a dose-dependent manner, with an EC_{50} of 20 nmol/l. The EC_{50} of the prototypical PARP inhibitor 3-aminobenzamide was 200 μmol/l. Peroxynitrite- and hydrogen peroxide–induced oxidation of dihydrorhodamine-123 was unaffected by PJ34 in the concentration range of 1 μmol/l to 10 mmol/l, indicating that the compound does not act as an antioxidant. Additional details of the synthesis and pharmacological characterization of PJ34 have been published previously (9).

Statistical analysis. Results are reported as means ± SE. Statistical significance between two measurements was determined by the two-tailed unpaired Student's t test, and among groups, it was determined by analysis of variance with Bonferroni's correction. Probability values of $P < 0.05$ were considered significant.

RESULTS

General characteristics of the animals. Progression of diabetes in NOD mice and after STZ injection in rats resulted in a decrease in the growth of the diabetic animals (Fig. 1). Body weight decreased by 16 and 35% by 5 weeks, and heart weight decreased by 12 and 26% by 5 weeks, thereby increasing the heart weight–to-body weight ratio by 5 and 13% in mice and rats, respectively. Diabetic mice and rats also exhibited increased serum concentrations of glucose and glycated hemoglobin (Fig. 1). Treatment of diabetic and control rats and mice with the PARP inhibitor PJ34 did not significantly influence body and heart weights or plasma levels of glucose and glycated hemoglobin. Pancreas insulin content (nanogram of insulin per milligram of pancreatic protein) was 40.3 ± 4.1 ($n = 10$), 45.9 ± 2.5 ($n = 7$), 1.9 ± 0.4 ($n = 14$), and 1.8 ± 0.3 ($n = 9$) in NOD control mice, NOD control mice treated with PJ34 for 4 weeks, NOD diabetic mice, and NOD diabetic mice treated with PJ34 for 4 weeks, respectively.

Ventricular function

Ventricular function in NOD mice. In untreated diabetic mice, heart rate, mean blood pressure, left ventricular systolic pressure, +dP/dt, and −dP/dt were significantly decreased, whereas left ventricular end-diastolic pressure increased. Treatment with PJ34 prevented the depression in left ventricular systolic pressure, +dP/dt, −dP/dt and the elevation of the left ventricular end-diastolic pressure (Fig. 2). PJ34 slightly improved the decreased heart rate but did not reverse the decrease in mean arterial blood pressure (Fig. 2) in diabetic mice. The PARP inhibitor treatment had no significant effects on hemodynamic parameters in control mice (Fig. 2).

Ventricular function in rats. Similar to mice, diabetes in rats was characterized by an increase in left ventricular end-diastolic pressure and a decrease in heart rate, mean

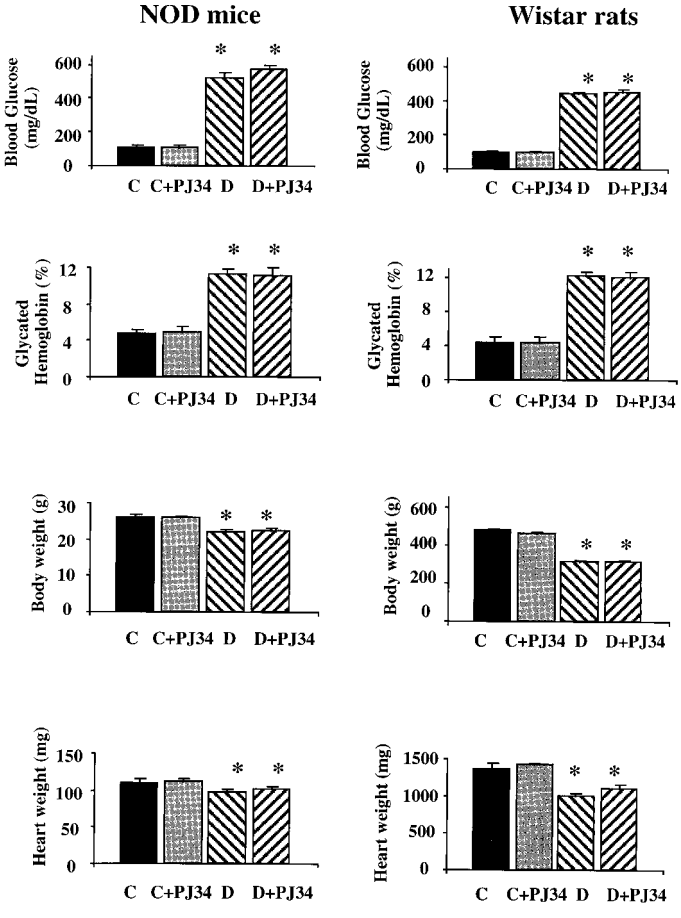

FIG. 1. General characteristics of animals. Body weights, heart weights, blood glucose, and glycated hemoglobin levels in control (C), PJ34-treated (for 4 weeks) control (C+PJ34), diabetic (D) (for 5 weeks), and PJ34-treated (for 4 weeks) diabetic (D+PJ34) NOD mice (on the left) and Wistar rats (on the right). In rats, diabetes was induced by STZ. Data are means ± SE. *P < 0.05 vs. C; #P < 0.05 vs. D.

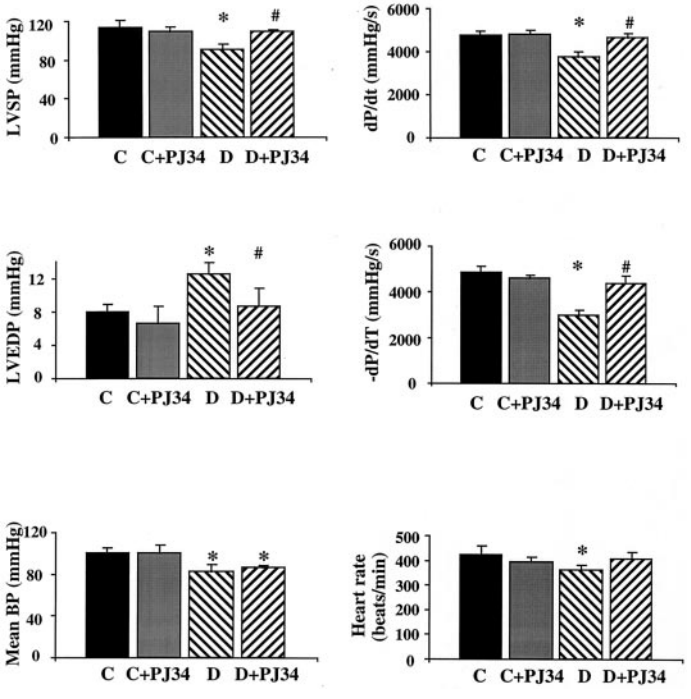

FIG. 2. Reversal of diabetes-induced cardiac dysfunction by pharmacological inhibition of PARP in an autoimmune mouse model of diabetes. Effect of diabetes and PJ34 on left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), left ventricular +dP/dt, left ventricular −dp/dt, mean blood pressure (mean BP), and heart rate in NOD mice. C, control; D, diabetic; C+PJ34, control treated with PJ34 (for 4 weeks); D+PJ34, diabetic treated with PJ34 (for 4 weeks). Data are means ± SE. *P < 0.05 vs. C; #P < 0.05 vs. D.

blood pressure, left ventricular systolic pressure, +dP/dt, and −dP/dt (Fig. 3). PJ34 treatment significantly improved the depression in left ventricular systolic pressure and diastolic −dP/dt. Moreover, the elevation of the left ventricular end-diastolic pressure and the decrease of systolic +dP/dt was completely prevented by the PARP inhibitor treatment. The PJ34 treatment also improved the decreased heart rate and mean blood pressure in diabetic rats. Similar to the findings in mice, PJ34 treatment in normal (nondiabetic) rats had no significant effects on hemodynamic parameters (Fig. 3).

In a separate set of experiments, diabetic rats (n = 12) were treated with PJ34 for 6 weeks followed by the discontinuation of the treatment for an additional 3-week period. The improved cardiac function in these rats was still maintained at 3 weeks after discontinuation of the treatment, when compared with the matched 10-week diabetic animals (Fig. 4).

Evidence for PARP activation in the diabetic hearts in vivo. As shown in Fig. 5, a marked degree of PARP activation was observed in the hearts isolated from 5- and 10-week-old diabetic rats. The staining was mainly localized in the nuclei of cardiac myocytes.

Similarly, immunohistochemical evidence of PARP activation was found in the hearts of the diabetic NOD mice (Fig. 6). In both rats and mice, the PARP activation was markedly attenuated by treatment with PJ34 (Figs. 5–6). The percentage of PARP-positive nuclei of myocytes was 1.7 ± 0.7% (n = 5) and 1.1 ± 0.3% (n = 5) in control nondiabetic rats and NOD mice, respectively. Five weeks of diabetes increased the fraction of PARP-positive nuclei to 23.6 ± 6% (n = 5, P < 0.01) and 21.6 ± 3.6% (n = 5, P < 0.01) in rats and NOD mice, respectively. Treatment with PJ34 (for 4 weeks) markedly decreased the percentage of PARP-positive nuclei to 4.3 ± 0.7% (n = 5, P < 0.01) and 3.3 ± 0.7% (n = 5, P < 0.01) in both rats and mice, respectively. At 10 weeks, in the hearts of the diabetic rats, the percentage of PARP-positive nuclei was 26.5 ± 5.5% (n = 5). Treatment with PJ34 (for 6 weeks) of diabetic rats even after discontinuation for 3 weeks reduced the percentage of PARP-positive nuclei to 7.1 ± 1.9% (n = 5, P = 0.01).

Diabetes induces a PARP-dependent endothelial dysfunction in NOD mice. Ex vivo experiments demonstrated the loss of endothelial function, as measured by the relaxant responsiveness of precontracted vascular rings to the endothelium-dependent vasodilator, nitric oxide–liberating hormone acetylcholine (Fig. 7). Inhibition of PARP activation was achieved by chronic treatment with the potent, water-soluble phenantridinone derivative PARP inhibitor PJ34, starting at 1 week after the onset of diabetes. This treatment restored normal vascular function (Fig. 7). The endothelium-independent relaxant response to sodium nitroprusside was unchanged (Fig. 7), indicating that the ability of the smooth muscle to relax to nitric oxide was not impaired in diabetes. The contractile

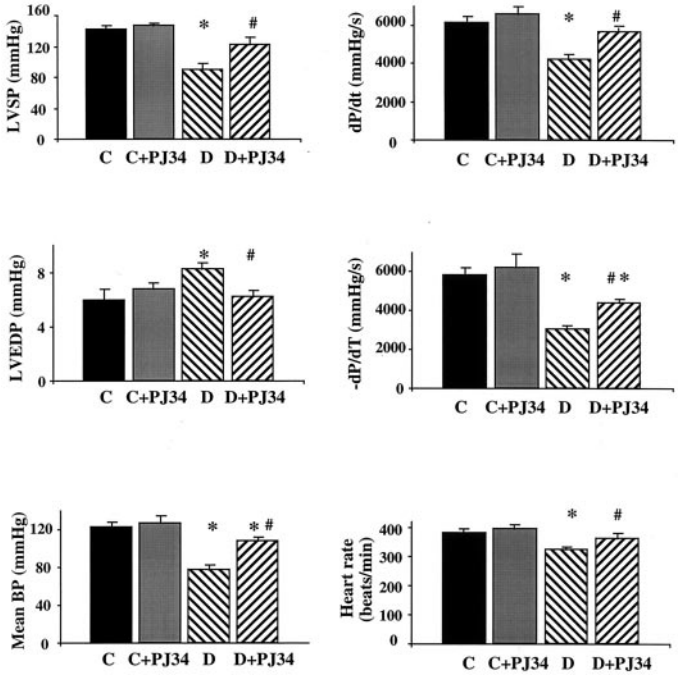

FIG. 3. Reversal of STZ-evoked diabetes induced cardiac dysfunction by pharmacological inhibition of PARP in rats. Effect of diabetes (5 weeks) and PJ34 (4 weeks) on left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), left ventricular +dP/dt, left ventricular −dp/dt, mean blood pressure (mean BP), and heart rate in rats. C, control; D, diabetic (for 5 weeks); C+PJ34, control treated with PJ34 (for 4 weeks); D+PJ34, diabetic treated with PJ34 (treatment was started after 1 week of established diabetes for further 4 weeks). Data are means ± SE. *P < 0.05 vs. C; #P <0.05 vs. D.

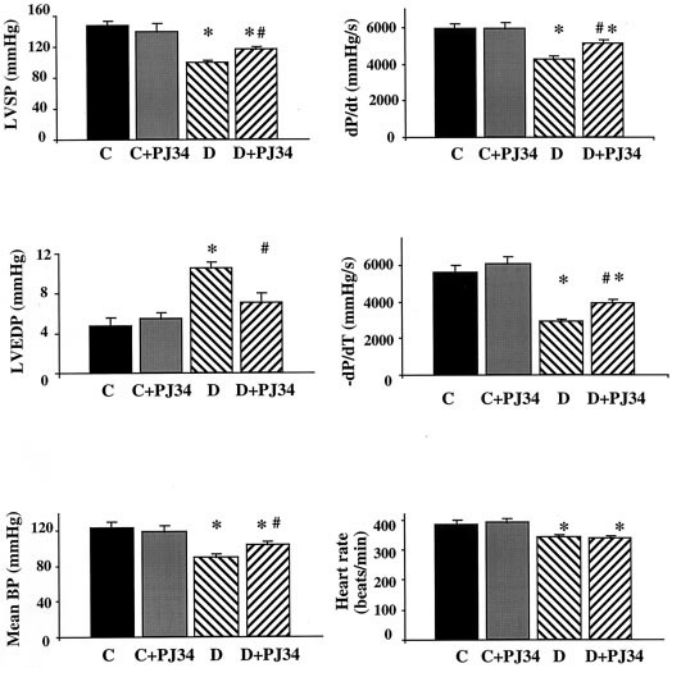

FIG. 4. Preservation of beneficial effects of pharmacological inhibition of PARP on cardiac dysfunction in STZ-induced diabetic rats after discontinuation of the treatment for 3 weeks. Effect of diabetes (10 weeks) and PJ34 on left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), left ventricular +dP/dt, left ventricular −dp/dt, mean blood pressure (mean BP), and heart rate in rats. C, control; D, diabetic; C+PJ34, control treated with PJ34 (PJ34 treatment was started after 1 week of established diabetes for 6 weeks followed by discontinuation for 3 weeks); D+PJ34, diabetic rat treated with PJ34 (for 6 weeks). Data are means ± SE. *P < 0.05 vs. C; #P < 0.05 vs. D.

responsiveness of the thoracic aorta to norepinephrine was unchanged in the diabetic NOD mice. PJ34 treatment had no significant effects on contractile or endothelium-dependent and -independent relaxant responses in control animals (Fig. 7).

DISCUSSION

Diabetic cardiomyopathy is characterized by complex changes in the mechanical, biochemical, structural, and electrical properties of the heart, which may be responsible for the development of an early diastolic dysfunction and increased incidence of cardiac arrhythmias in diabetic patients. Despite the expanding knowledge obtained from different models of diabetes, the mechanism of diabetic cardiac diastolic dysfunction remains elusive (rev. in 14,15,18–20).

The present study demonstrates that both genetic and STZ-induced diabetes in NOD mice and rats were associated with a marked depression of left ventricular function involving both systolic pressure development and relaxation. These results are in agreement with earlier reports showing depressed cardiac performance in different mouse (21,22) and rat (13,23–27) models of diabetes. Importantly, the results presented here document for the first time that in both genetic and STZ-induced models of type 1 insulin-dependent diabetes, the impaired cardiac function is associated with an activation of PARP in the myocardium. Furthermore, we now show that the diabetic cardiac dysfunction can be reversed by pharmacological inhibition of PARP.

Clinical and experimental investigations suggested that increased sympathetic activity, activated cardiac renin-angiotensin system, myocardial ischemia/functional hypoxia, and elevated circulating levels of glucose result in oxidative stress. Oxidative stress associated with an impaired antioxidant defense status may play a critical role in subcellular remodeling, calcium-handling abnormalities, and subsequent diabetic cardiomyopathy (21,28–30). Moreover, recent studies have suggested that oxidative damage may be critical in the early onset of diabetic cardiomyopathy (21,30). Consistent with this idea, significant nitrotyrosine formation was reported in cardiac myocytes from myocardial biopsy samples obtained from diabetic and diabetic-hypertensive patients (30) and in a mouse model of STZ-induced diabetes (21). Superoxide anion interacts with nitric oxide, forming the oxidant peroxynitrite ($ONOO^-$), which attacks various biomolecules, leading to—among other things—the production of a modified amino acid, nitrotyrosine (31). Although nitrotyrosine was initially considered a specific marker of peroxynitrite generation, other pathways can also induce tyrosine nitration (32). Thus, nitrotyrosine is now generally considered a collective index of reactive nitrogen species, rather than a specific indicator of peroxynitrite formation (32,33). Nevertheless, the increase in nitrotyrosine in myocytes with diabetes (21,30) suggested that a causative link exists between nitrosative stress and the disease. Oxidative and nitrosative stress in diabetic hearts is accompanied by increased formation of hydrogen peroxide and peroxynitrite, which are endogenous inducers of DNA single-strand breakage (21,30). DNA single-strand

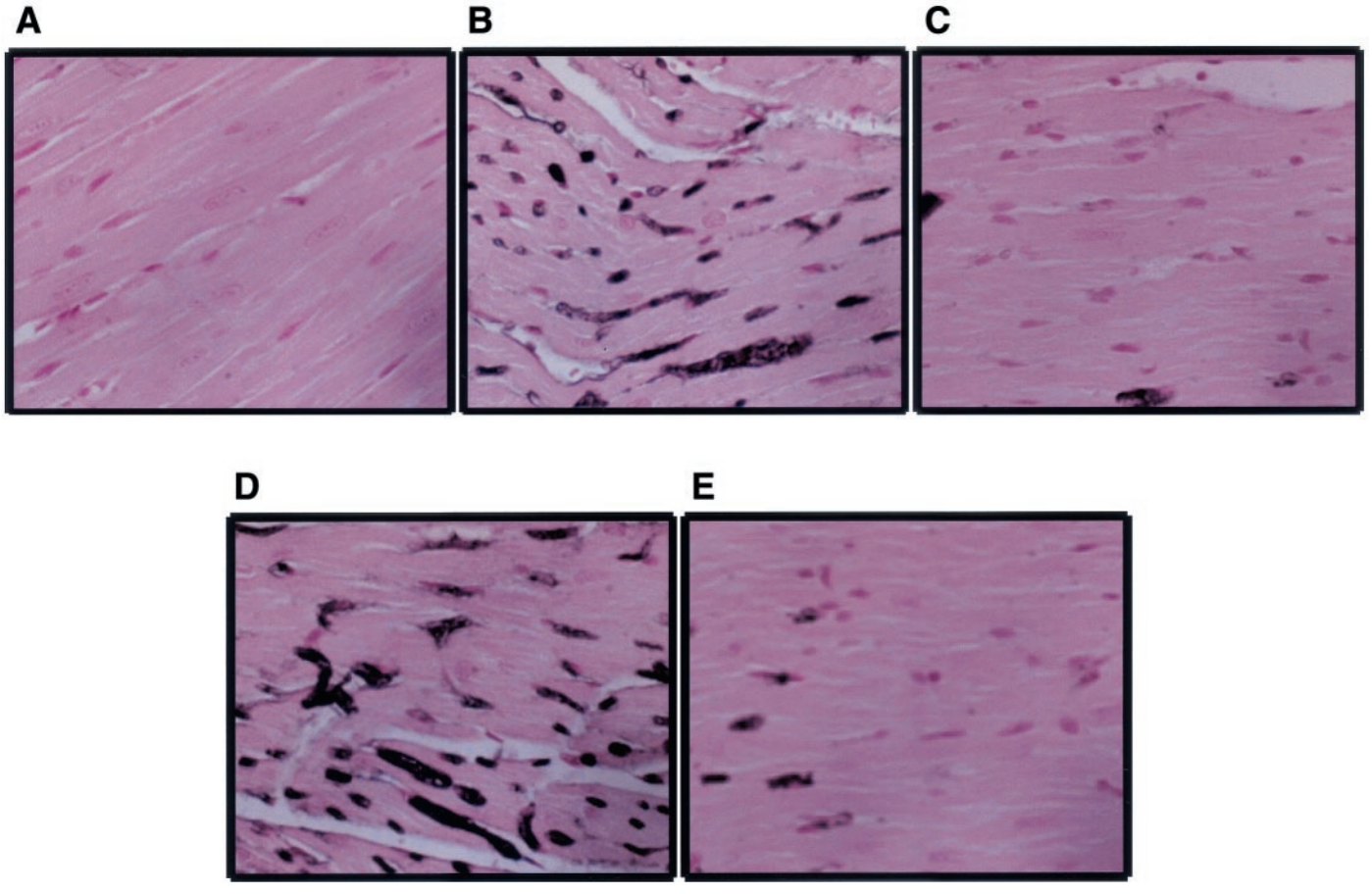

FIG. 5. Evidence for PARP activation in diabetic rat hearts. Immunohistochemical staining for poly(ADP-ribose) formation, an indicator of PARP activation, in control (*A*), diabetic (*B* and *D*), and PJ34-treated diabetic (*C* and *E*) rat hearts. Panels *B* and *D* show poly(ADP-ribose) formation localized in the nuclei of myocytes in 5- and 10-week diabetic rat hearts, respectively. The evidence of poly(ADP-ribose) accumulation can be seen as dark, frequent, and widespread nuclear staining in panels *B* and *D*. Treatment with PJ34 for 4 weeks (*C*) markedly reduced PARP activation in diabetic (5-week) hearts. Notably, the PARP activation in diabetic hearts (10 weeks) was attenuated even after the discontinuation of the treatment with PJ34 (after 6 weeks) for an additional 3-week period (*E*). Similar immunohistochemical profiles were seen in $n = 4–5$ hearts per group.

breakage is the obligatory trigger of PARP activation (1,2,7,34), which in turn may result in rapid depletion of the intracellular NAD^+ and ATP pools, thus slowing the rate of glycolysis and mitochondrial respiration, which eventually leads to cellular dysfunction and death. The importance of the PARP pathway is well documented in various models of myocardial ischemia-reperfusion injury (another condition in which oxidative stress plays a key pathogenetic role) (4,35–42). Based on the results of the current study, we conclude that the "reactive oxygen/nitrogen species–DNA injury–PARP activation" pathway also plays a pathogenetic role in the development of diabetic cardiomyopathy.

In recent studies (9,10) we have demonstrated that in vitro and in vivo endothelial cell dysfunction in response to high glucose is associated with increased poly(ADP-ribos)ylation. Furthermore, endothelial function is maintained in vascular rings of animals in which PARP-1 is inactivated or PARP is pharmacologically inhibited. In the present study, we extended these findings by showing that the genetically diabetic NOD mice also develop severe endothelial dysfunction, which can be reversed by PARP inhibitor treatment. These findings further strengthen the view that the activation of PARP is an important factor in the pathogenesis of endothelial dysfunction in diabetes. We have also measured cellular nucleoside levels in endothelial cells exposed to high glucose, as well as in diabetic vascular rings, in the presence or absence of PJ34 treatment (9,10). It was observed that diabetes in vivo (10) and high glucose incubation in vitro (9) induces a severe metabolic suppression of the endothelial cells and of the blood vessels, characterized by NAD, NADPH, and ATP depletion, effects that could be partially restored by pharmacological inhibition of PARP (9,10). If similar mechanisms are operative in the diabetic hearts, it is conceivable that—in addition to a variety of other mechanisms—a direct energetic deficit may also contribute to the depression of myocardial contractility.

It is possible that the diabetic endothelial PARP pathway and the diabetic cardiomyopathy are interrelated: an impairment of the endothelial function may lead to global or regional myocardial ischemia, which may secondarily impair cardiac performance (43,44). It is noteworthy that the protective effect of PARP inhibition against diabetic cardiac dysfunction extended several weeks beyond the discontinuation of treatment; this observation may have important implications for the design of future clinical trials with PARP inhibitors. We have determined the

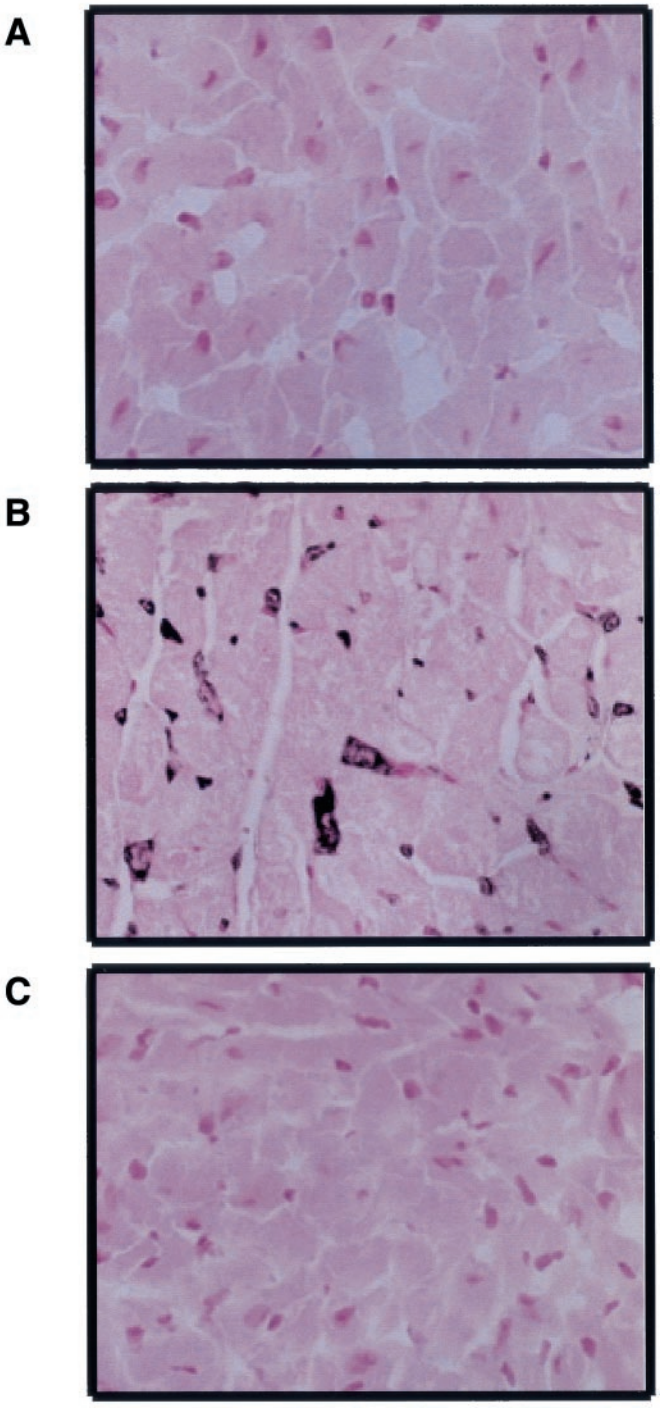

FIG. 6. Evidence for PARP activation in diabetic mouse hearts. Immunohistochemical staining for poly(ADP-ribose) formation, an indicator of PARP activation, in control (*A*), diabetic (*B*), and PJ34-treated diabetic (*C*) mouse hearts. The evidence of enhanced poly(ADP-ribose) activity can be seen as dark, frequent, and widespread nuclear staining in panel *B*. Similar immunohistochemical profiles were seen in $n = 4–5$ hearts per group.

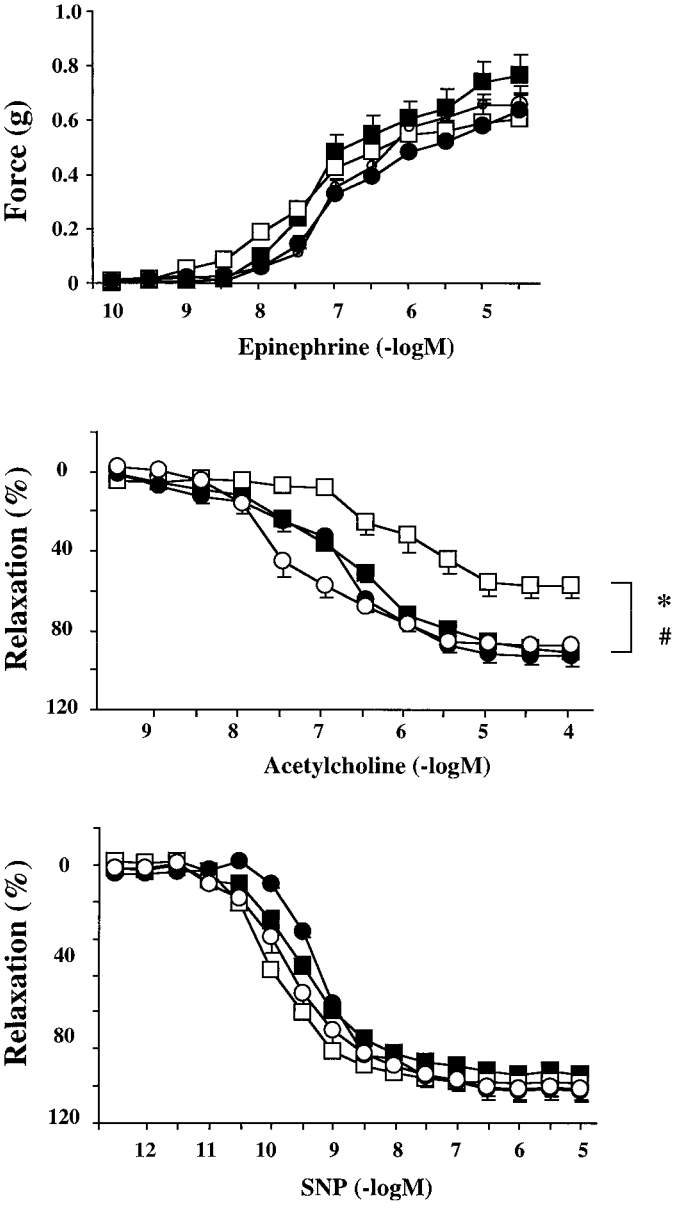

FIG. 7. Reversal of diabetes-induced endothelial dysfunction by pharmacological inhibition of PARP in diabetic NOD mouse vascular rings. Epinephrine-induced contractions (upper panel), acetylcholine-induced endothelium-dependent relaxation (middle panel), and sodium nitroprusside–induced endothelium-independent relaxations (lower panel). ■, control; ○, control + PJ34; □, diabetes; ●, diabetes + PJ34. Each point of the curve represents the means ± SE of 5–8 experiments in vascular rings. *$P < 0.05$ vs. C; #$P < 0.05$ vs. D.

pharmacokinetic profile of intravenously injected PJ34 in rats, and we found that the compound has a plasma half-life of ~2 h. No detectable PJ34 was found 24 h after the single intravenous injection of PJ34 in the animals (G.J. Southan, C.S., unpublished data). These observations support the view that the prolonged persistence of the effect of PJ34 is not related to the continued presence of the inhibitor, but, rather, may be related to the permanent interruption by the PARP inhibitor of positive feedback cycles of cardiac injury. 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 intracellular oxidant generation (as discussed in 2,34,37,47). It is also conceivable that the degree of PARP activation may be more pronounced at the onset of the development of diabetic cardiovascular complications (as compared with a later stage of the disease) and when PARP is inhibited at an earlier time; this may result in more sustained beneficial effects. Although we cannot determine from the current studies whether the delayed

protection is related to a downregulation of the triggers of PARP activation (such as oxidant production), or whether it may be related to the time course of the phenomenon, it is clear that even after the discontinuation of the treatment with the PARP inhibitor, only a very low level of PARP activation was seen in the myocardial sections (Fig. 5*E*).

One may raise the issue whether the loss of body weight (due to insulin deficiency) seen in our models of diabetes has contributed to the development of cardiomyopathy. For the following reasons, we feel that it is unlikely that the 35 and 16% reductions of body weight in rats and mice could solely account for the characteristic diabetic cardiac dysfunction described in our models: *1*) In a recent study by Kajstura et al. (21), using similar functional measurements, a similar degree of cardiac diastolic and systolic dysfunction was demonstrated in a mouse STZ diabetes model. Kajstura et al. also used restricted diet in control animals to match the decrease in body weight with diabetes. The characteristic diabetic cardiac dysfunction was evident at 1 week after STZ administration and was more pronounced after 30 days, thus it could not be attributed to the body weight decrease associated with the diabetes. *2*) In addition, it is also unlikely that the beneficial effect of PJ34 on myocardial function is associated with an anabolic effect since PJ34 treatment did not influence the body and heart weight loss in diabetic animals, whereas it dramatically improved cardiac function.

Although a cause-effect relationship has not been determined in the current study, it is conceivable that some of the alterations in the cardiac performance reported in the current study may be, at least in part, related to alterations in blood pressure.

The role of PARP activation in diabetes is not limited to the vascular dysfunction. A vast body of evidence supports the role of PARP activation in the primary process of diabetes, i.e., in the process of islet cell destruction. Pretreatment of the animals with PARP inhibitors known to protect against STZ-induced β-cell necrosis and hyperglycemia (45–47). Similarly, PARP-deficient mice are resistant against STZ-induced diabetes (5,6,48). However, to effectively prevent the primary process of islet cell death, PARP inhibitors must be applied in a pretreatment regimen. PARP inhibitors rapidly lose their effectiveness in protecting the primary process of diabetic islet cell death, when their administration is delayed relative to the induction of diabetes (5,6). In fact, this phenomenon has been utilized to our advantage in the current experimental design: the start of the PARP inhibitor administration was delayed to the time when the primary islet cell destruction was complete in order to prevent its interference with the development of diabetic islet injury and subsequent hyperglycemia.

In conclusion, our study provides experimental evidence that the PARP plays a central role in the pathogenesis of diabetic cardiovascular dysfunction. Further work is required to clarify whether PARP inhibition may exert beneficial effects against the development of various cardiovascular complications in diabetic patients.

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