

# Activation of poly(ADP-ribose) polymerase contributes to the endothelial dysfunction associated with hypertension and aging

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**Abstract.** Increased production of reactive oxygen and nitrogen species has recently been implicated in the pathogenesis of endothelial dysfunction associated with atherosclerosis, hypertension and aging. Oxidant induced cell injury triggers the activation of nuclear enzyme poly(ADP-ribose) polymerase (PARP), which in turn contributes to cardiac and vascular dysfunction in various pathophysiological conditions including diabetes, reperfusion injury and circulatory shock. Here we investigated the role of PARP activation in the pathogenesis of cardiac and endothelial dysfunction associated with atherosclerosis, hypertension and aging. Retired breeder spontaneously hypertensive rats (SHR, 40 weeks old) and apolipoprotein E knockout mice (apoE-Ko, 10 weeks old) were treated for 20 weeks with vehicle or the potent PARP inhibitor PJ34. In the vehicle-treated SHR rats and apoE-Ko mice (kept on atherogenic diet) there was a significant loss of endothelial function, as measured by the relaxant responsiveness of vascular rings to acetylcholine. SHR rats also developed severe hypertension and cardiac hypertrophy. Treatment with the PARP inhibitor did not influence high blood pressure and cardiac hypertrophy in SHR rats, but it improved Ach-induced, NO-mediated vascular relaxation. In addition to the beneficial effects of chronic treatment with PARP inhibitor, 1-h *in vitro* incubation of aortic rings from SHR rats with PJ34 (3  $\mu\text{mol/l}$ ) was also able to improve the endothelial dysfunction. In contrast, in apoE-Ko mice PJ34 treatment did not affect the parameters studied. Thus, PARP activation contributes to the pathogenesis of endothelial dysfunction associated with

hypertension and aging, but not in the current experimental model of atherosclerosis.

## Introduction

Impaired endothelial function has been widely described both in animal models and in humans in atherosclerosis, hypertension and aging. There is circumstantial evidence that the endothelial dysfunction associated with these conditions is, at least in part, related to the local formation of reactive oxygen and nitrogen species within and in the vicinity of the vascular endothelium (1-18). Oxidant induced cell injury triggers the activation of nuclear enzyme poly(ADP-ribose) polymerase (PARP) leading to endothelial dysfunction in various pathophysiological conditions including reperfusion, shock, diabetes (19-26). Here we investigated whether the loss of endothelial function associated with hypertension and aging in rats and atherosclerosis in mice is dependent upon the PARP pathway within the vasculature.

## Materials and methods

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by US 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.

*Animals, treatment protocols.* Retired ex-breeder male spontaneous hypertensive rats (SHR, 40 weeks old) were treated with vehicle (n=15) or the PARP inhibitor PJ34 (n=15; 20 mg/kg/day PO) for 20 weeks. This dose regimen was found to effectively inhibit vascular PARP activation in previous studies (24-26). Eight weeks old male Wistar-Kyoto (WKY) rats treated with vehicle (n=15) or PJ34 (n=15; 20 mg/kg/day PO) for 20 weeks were used as controls.

Apolipoprotein E knockout mice (apoE-Ko, 10 weeks old) and age-matched controls were kept on atherogenic diet (ICN Biomedicals Inc., Aurora, OH) and were treated for 20 weeks with vehicle or the PARP inhibitor PJ34.

*Measurement of vascular reactivity in isolated aortic rings of rats and mice.* The method has been described previously (24-26). Briefly, the thoracic aorta was cleared from periadventitial fat and cut into 1-2 mm (in mice) or 3-4 mm (in rats) width

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*Abbreviations:* PARP, poly(ADP-ribose) polymerase; BP, blood pressure; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; +dP/dt, maximal slope of systolic pressure increment; -dP/dt, maximal slope of diastolic pressure decrement; SHR, spontaneous hypertensive rats

*Key words:* hypertension, aging, atherosclerosis, vasorelaxation, acetylcholine, oxidative stress, blood pressure

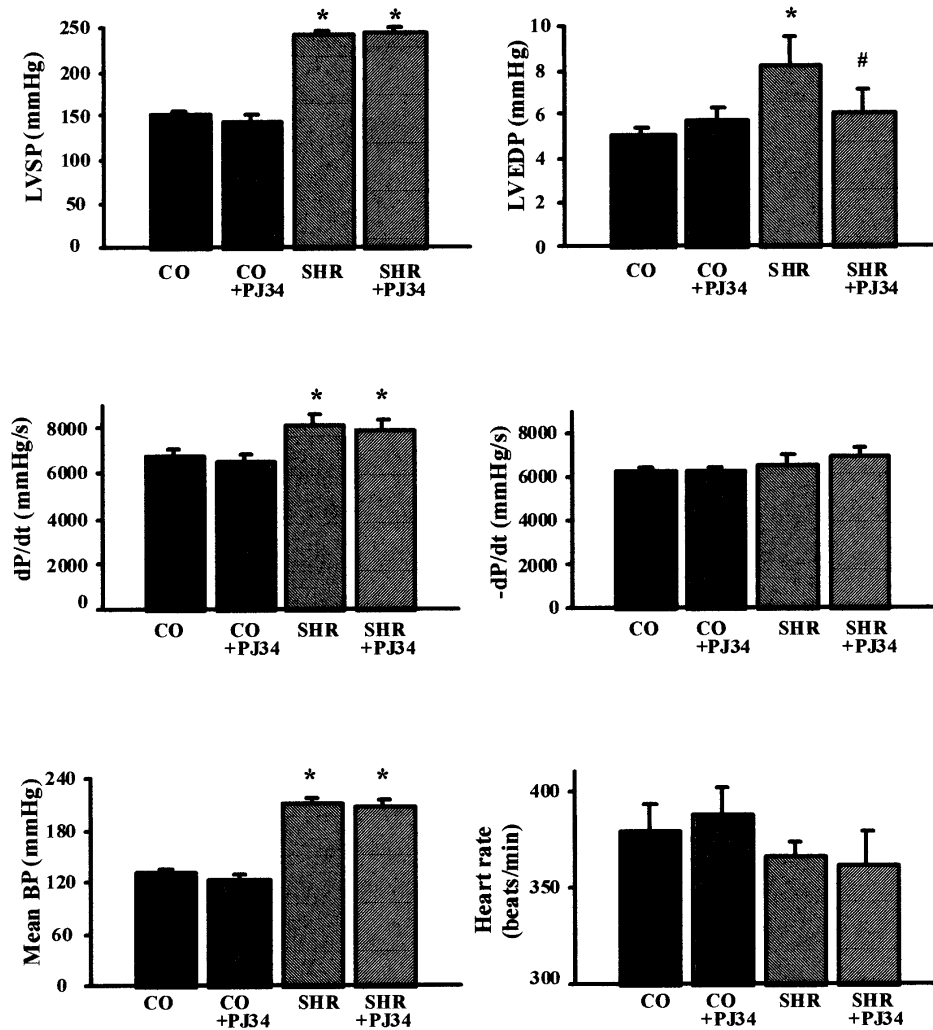


Figure 1. The effects of hypertension (SHR) and pharmacological inhibition of PARP on cardiac function. Effect of hypertension and PJ34 on left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), left ventricular +dP/dt, left ventricular -dP/dt, mean BP and heart rate in rats. CO, control; SHR, spontaneous hypertensive rats (15 months old); CO + PJ34, control treated with PJ34 (for 5 months); SHR + PJ34, spontaneous hypertensive rats treated with PJ34 (for 5 months). Results are mean  $\pm$  SEM of 7-10 experiments in each group. \* $P < 0.05$  vs. CO; # $P < 0.05$  vs. SHR.

rings using operation microscope, mounted in organ baths filled with warmed ( $37^{\circ}\text{C}$ ) and oxygenated (95%  $\text{O}_2$ , 5%  $\text{CO}_2$ ) Krebs' solution ( $\text{CaCl}_2$  1.6 mM;  $\text{MgSO}_4$  1.17 mM; EDTA 0.026 mM;  $\text{NaCl}$  130 mM;  $\text{NaHCO}_3$  14.9 mM;  $\text{KCl}$  4.7 mM;  $\text{KH}_2\text{PO}_4$  1.18 mM; glucose 11 mM). 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 (in mice) or 1.5 g (in rats) was applied and the rings were equilibrated for 60 min, followed by measurements of the concentration-dependent contraction to epinephrine ( $10^{-10}$  to  $3 \times 10^{-5}$  M), and in rings precontracted with epinephrine ( $10^{-6}$  M), relaxation to acetylcholine ( $10^{-9}$  to  $3 \times 10^{-4}$  M) and sodium nitroprusside ( $10^{-12}$ - $10^{-5}$  M). Experiments were conducted in 8-10 pairs of rings in each experimental group.

In a separate set of experiments rings from the control or hypertensive animals were incubated with PJ34 (3  $\mu\text{mol/l}$ ) or vehicle for 1 h, followed by the determination of endothelium-dependent vascular function.

*Hemodynamic measurements in rats.* Analysis of left ventricular performance was measured in rats anesthetized with i.p. injection of thiopentone sodium (60 mg  $\text{kg}^{-1}$ ). Animals were placed on controlled heating pads, and core temperature measured via a rectal probe was maintained at  $36$ - $38^{\circ}\text{C}$ . A microtip catheter transducer (SPR-524; Millar Instruments, Houston, TX, USA) 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 (LVSP and LVEDP) were measured and the maximal slope of systolic pressure increment (+dP/dt) and diastolic pressure decrement (-dP/dt), an 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. The method was described previously in detail (26). After the hemodynamic measurements were made, animals were sacrificed by lethal injection of thiopentone sodium.

Hemodynamic measurements were conducted in 7-10 animals in each group.

**Statistical analysis.** Results are reported as mean  $\pm$  SEM. 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.

**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 (24).

## Results

**Cardiac remodeling.** Following the hemodynamic measurements, the heart was removed and left and right ventricles were dissected and weighed. The weight of each ventricle was then normalized to the body weight of the animals. In hypertensive aging animals the left and right ventricular weights normalized to body weight (LVW/BW, RVW/BW: g heart weight/kg body weight) were significantly increased as compared to the controls [ $1.76 \pm 0.04$  (control;  $n=9$ ) vs.  $3.71 \pm 0.17$  (SHR;  $n=10$ ) and  $0.44 \pm 0.01$  (control;  $n=9$ ) vs.  $0.74 \pm 0.11$  (SHR;  $n=10$ ), respectively]. PJ34 treatment for 5 months did not influence the LVW/BW, RVW/BW ratio (g/kg) of control [ $1.76 \pm 0.04$  (control;  $n=9$ ) vs.  $1.68 \pm 0.05$  (control + PJ34;  $n=9$ ) and  $0.44 \pm 0.01$  (control;  $n=9$ ) vs.  $0.43 \pm 0.01$  (control + PJ34;  $n=9$ )] and of aging hypertensive animals [ $3.71 \pm 0.17$  (SHR;  $n=10$ ) vs.  $3.33 \pm 0.12$  (SHR + PJ34;  $n=9$ ); and  $0.74 \pm 0.11$  (SHR;  $n=10$ ) vs.  $0.62 \pm 0.04$  (SHR + PJ34;  $n=9$ )], respectively.

**Ventricular function in rats.** In hypertensive aging rats the LVSP, mean BP, LVEDP and systolic dP/dt was significantly ( $P < 0.01$ ) elevated as compared to control animals, while there was no change in the diastolic -dP/dt and heart rate (Fig. 1). PJ34 treatment did not significantly influence the increase in LVSP, mean BP, and systolic dP/dt, however, significantly decreased the elevated LVEDP in hypertensive aging animals. PJ34 treatment in control rats had no significant effects on hemodynamic parameters (Fig. 1).

**Survival of animals.** During the treatment period of 5 months 4 vehicle-treated SHR rats died, which developed nasal bleeding and stroke. In contrast, all animals treated with PJ34 were alive at the end of the treatment period. The beneficial effects of PARP inhibitors in stroke were previously demonstrated (27,28).

In the apoE-Ko mice on atherogenic diet we lost 5 animals in vehicle treated group and 6 in PJ34-treated one during the 5 months treatment period, while all the control animals fed on high fat diet survived.

**Hypertension and aging induces a PARP-dependent endothelial dysfunction in rat.** *Ex vivo* experiments demonstrated the loss

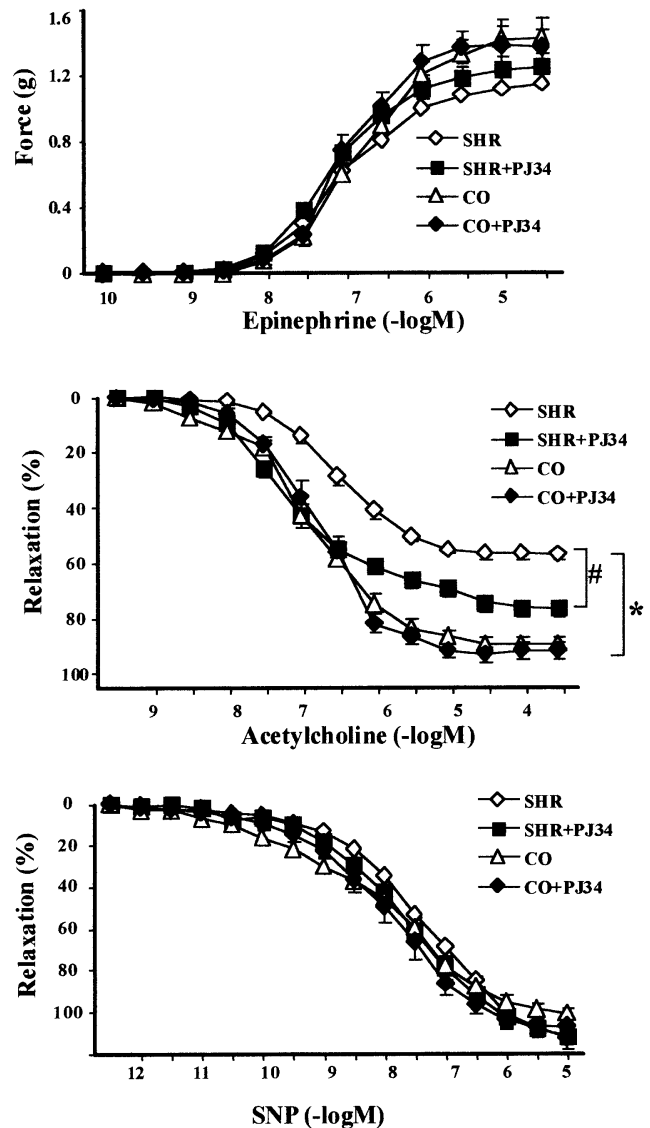


Figure 2. Chronic treatment (for 5 months) by pharmacological inhibitor of PARP improves hypertension-induced endothelial dysfunction in rats. Epinephrine-induced contractions (upper panel), Ach-induced endothelium-dependent relaxation (middle panel) and SNP-induced endothelium-independent relaxations (lower panel). Each point of the curve represents mean  $\pm$  SEM of 8-10 experiments in vascular rings. \* $P < 0.05$  vs. control; # $P < 0.05$  vs. SHR.

of endothelial function, as measured by the relaxant responsiveness of pre-contracted vascular rings to the endothelium-dependent vasodilator, NO liberating hormone acetylcholine (Ach) in aging hypertensive rats (15 months old) (Fig. 2). In contrast to the 15 months old SHR rats aortic rings taken from 15 months old WKY rats ( $n=5$ ) did not show impaired endothelium-dependent dilatory response to Ach (data not shown). Inhibition of PARP activation was achieved by chronic treatment with the potent, water-soluble phenanthridinone derivative PARP inhibitor PJ34 for 5 months. This treatment significantly improved vascular function (Fig. 2) in hypertensive aging animals. The endothelium-independent relaxant response to sodium nitroprusside was unchanged (Fig. 2), indicating the ability of the endothelium to release NO, rather than the ability of the smooth muscle to relax to

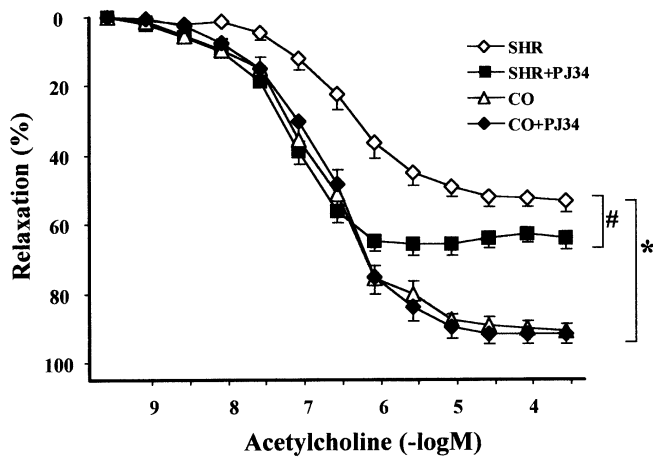


Figure 3. *In vitro* incubation of aortic rings by pharmacological inhibitor of PARP improves hypertension-induced endothelial dysfunction in rats. Ach-induced endothelium-dependent relaxation from aortic rings in control and 15 months old SHR rats incubated with 3  $\mu$ M PJ34 or vehicle for 1 h. Each point of the curve represents mean  $\pm$  SEM of 8-10 experiments in vascular rings. \* $P$ <0.05 vs. control; # $P$ <0.05 vs. SHR.

NO is impaired in hypertensive aging rats. The contractile responsiveness of the thoracic aorta in aging hypertensive rats was not significantly changed (Fig. 2) as compared to controls. PJ34 treatment had no significant effects on contractile or endothelium-dependent and independent relaxant responses in control animals (Fig. 2). Surprisingly, local incubation of aortic rings from 15 months old SHR rats with PARP inhibitor, PJ34, for 1 h also significantly improved endothelium-dependent relaxation responses to Ach (Fig. 3). Local incubation of aortic rings obtained from control animals with PJ34 did not change endothelium-dependent relaxation responses to Ach (Fig. 3).

*Endothelial dysfunction in apoE-Ko mice is independent of PARP activation.* Apolipoprotein E knockout mice (apoE-Ko) kept on high fat atherogenic diet for 5 months developed severe endothelial dysfunction characterized by decreased sensitivity to Ach (Fig. 4). In contrast to endothelium-dependent relaxant response to Ach, the contractile response to epinephrine and endothelium-independent relaxation responses to SNP were not impaired in apoE-Ko mice kept on high fat diet (Fig. 4). The treatment with PARP inhibitor PJ34 for 5 months failed to improved vascular function in apoE-Ko mice. PJ34 treatment had no significant effects on contractile or endothelium-dependent and independent relaxant responses in control animals (Fig. 4).

## Discussion

The endothelial dysfunction in hypertension, aging and atherosclerosis is well characterized both in animal models and humans (8,10-14,17,29-35). The impairment of vascular relaxation in hypertension, aging and atherosclerosis is, at least in part, related to the increased local formation of reactive oxygen and nitrogen species within and in the vicinity of the vascular endothelium (1-18). Superoxide anion interacts with nitric oxide, forming the oxidant peroxynitrite (ONOO), which

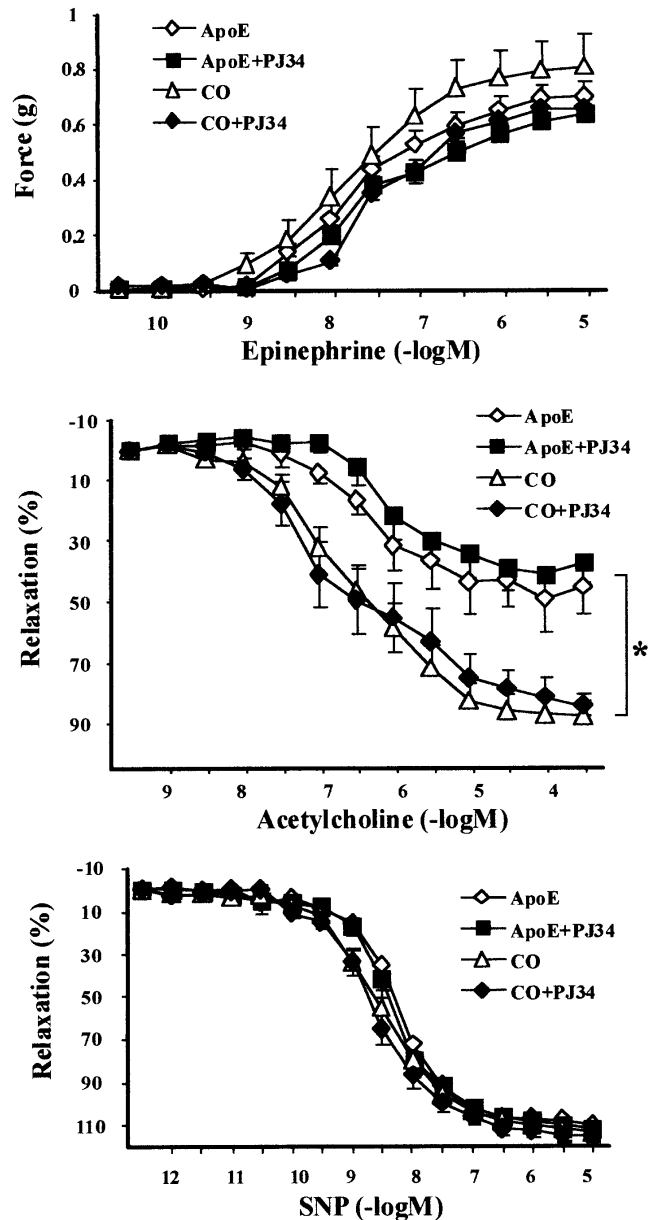


Figure 4. Chronic treatment (for 5 months) by pharmacological inhibitor of PARP fails to improve endothelial dysfunction in ApoE-Ko mice fed on atherogenic diet. Epinephrine-induced contractions (upper panel), Ach-induced endothelium-dependent relaxation (middle panel) and SNP-induced endothelium-independent relaxations (lower panel). Each point of the curve represents mean  $\pm$  SEM of 8-10 experiments in vascular rings. \* $P$ <0.05 vs. control; # $P$ <0.05 vs. ApoE-Ko.

attacks various biomolecules, leading - among others - to the production of a modified amino acid nitrotyrosine (36). Although nitrotyrosine was initially considered a specific marker of peroxynitrite generation, other pathways can also induce tyrosine nitration (37). Thus, nitrotyrosine is now generally considered a collective index of reactive nitrogen species, rather than a specific indicator of peroxynitrite formation (37,38). Indeed, increased nitrotyrosine formation was reported in the vasculature of hypertensive and aging animals (11,16,39).

Oxidative stress accompanied by increased formation of hydrogen peroxide, superoxide anion and peroxynitrite are

endogenous inducers of DNA single strand breakage and DNA single strand breakage is the obligatory trigger of PARP activation (40), which in turn may result 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 necrosis. The protective effect of pharmacological inhibition of PARP or lack of PARP gene in preventing vascular dysfunction has been demonstrated in experimental models of shock, reperfusion injury, reperfusion injury after heart transplantation, diabetes and diabetic complications (other conditions where oxidative stress plays a key pathogenetic role) (19-26,41-49).

The present study demonstrates that the chronic treatment with PARP inhibitor PJ34 significantly improves the impaired endothelium-dependent dilatory response to Ach in aortic rings from 15 months old hypertensive aging rats (Fig. 2). Furthermore, we show that the local incubation with PARP inhibitor, PJ34, for 1 h also improves Ach-induced endothelium-dependent relaxant response in hypertensive aging rats (Fig. 3). This finding resembles our recent observations in diabetic vasculature, where *in vivo* treatment with PJ34 prevents the development of endothelial dysfunction, and *in vitro* incubation with the PARP inhibitor improves vascular function (25). In hypertensive aging rats LVSP, mean BP, LVEDP and systolic dP/dt was significantly elevated as compared to control animals, while there was no change in the diastolic -dP/dt and heart rate (Fig. 1). The PARP inhibitor treatment did not significantly influence the increase in LVSP, mean BP, and systolic dP/dt, however, significantly decreased the elevated LVEDP. Hypertension was also characterized with ventricular hypertrophy of the heart (especially left ventricle), which was not significantly affected by PJ34 treatment.

In contrast to results obtained in hypertensive aging rats, we also show that PARP inhibition failed to affect endothelial dysfunction in a model of severe atherosclerosis in apoE-Ko mice fed on high fat diet.

In conclusion, our results show that the reactive oxygen/nitrogen species - PARP pathway plays a pathogenetic role in the development of endothelial dysfunction in a rat model of hypertension and aging, but not in the current model of atherosclerosis.

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