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Preconditioning protects the heart in a prolonged uremic condition

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[1]*Cardiovascular Research Group, Department of Biochemistry, Faculty of Medicine, University of Szeged, Szeged, Hungary;* [2]*Pharmahungary Group, Szeged, Hungary; and* [3]*Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary*

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Kocsis GF, Sárközy M, Bencsik P, Pipicz M, Varga ZV, Pálóczi J, Csonka C, Ferdinandy P, Csont T. Preconditioning protects the heart in a prolonged uremic condition. *Am J Physiol Heart Circ Physiol* 303: H1229–H1236, 2012. First published September 14, 2012; doi:10.1152/ajpheart.00379.2012.— Metabolic diseases such as hyperlipidemia and diabetes attenuate the cardioprotective effect of ischemic preconditioning. In the present study, we examined whether another metabolic disease, prolonged uremia, affects ischemia/reperfusion injury and cardioprotection by ischemic preconditioning. Uremia was induced by partial nephrectomy in male Wistar rats. The development of uremia was verified 29 wk after surgery. Transthoracic echocardiography was performed to monitor cardiac function. At *week 30*, hearts of nephrectomized and sham-operated rats were isolated and subjected to a 30-min coronary occlusion followed by 120 min reperfusion with or without preceding preconditioning induced by three intermittent cycles of brief ischemia and reperfusion. In nephrectomized rats, plasma uric acid, carbamide, and creatinine as well as urine protein levels were increased as compared with sham-operated controls. Systolic anterior and septal wall thicknesses were increased in nephrectomized rats, suggesting the development of a minimal cardiac hypertrophy. Ejection fraction was decreased and isovolumic relaxation time was shortened in nephrectomized rats demonstrating a mild systolic and diastolic dysfunction. Infarct size was not affected significantly by nephrectomy itself. Ischemic preconditioning significantly decreased infarct size from 24.8 ± 5.2% to 6.6 ± 1.3% in the sham-operated group and also in the uremic group from 35.4 ± 9.5% to 11.9 ± 3.1% of the area at risk. Plasma ANG II and nitrotyrosine were significantly increased in the uremic rats. We conclude that although prolonged experimental uremia leads to severe metabolic changes and the development of a mild myocardial dysfunction, the cardioprotective effect of ischemic preconditioning is still preserved.

chronic renal failure; myocardium; ischemic preconditioning; infarct size; myocardial function

ISCHEMIC PRECONDITIONING IS a well-characterized endogenous adaptive response of the myocardium in which brief cycles of ischemia markedly enhance the ability of the heart to withstand a subsequent ischemic injury (15). Although preconditioning confers remarkable cardioprotection in a variety of species (15, 44), including humans (21, 49, 55), we and others have shown that its effectiveness is attenuated by some risk factors and comorbidities such as metabolic diseases including hyperlipidemia (14–16) and diabetes (35, 54) both in animal models and humans (15).

Increasing prevalence of hyperlipidemia and diabetes in the aging population results in a dramatic rise in the prevalence of chronic kidney disease characterized by severe metabolic changes generally termed as uremia. The majority of patients suffering from uremia remain unaware of their condition for several years until reaching late stages of chronic kidney disease (5, 37). Uremia and especially end-stage renal failure have been shown to increase the risk of cardiovascular morbidity and mortality (18, 24). The prevalence of coronary artery disease at the start of dialysis is ~40% (53). In fact, cardiovascular disease is the main cause of death in patients with chronic renal failure (18, 50). The incidence of myocardial infarction is high in dialysis patients, and the outcome after myocardial infarction is poor (2). Left ventricular dysfunction is notable in a significant number of patients on chronic dialysis, especially after acute myocardial infarction (2, 19, 26). One may speculate that the metabolic changes seen in uremia may interfere with endogenous cardioprotective mechanisms as seen with other metabolic diseases. However, there are limited data on the possible interaction of uremia with endogenous adaptive cardioprotective mechanisms including pre- and postconditioning.

A preliminary study from our research group has previously demonstrated for the first time in the literature that the infarct size limiting effect of ischemic postconditioning is still preserved in experimental chronic renal failure 10 wk after subtotal nephrectomy (32). Byrne et al. (4) has recently reported that ischemic preconditioning, remote ischemic conditioning, and ischemic postconditioning are still cardioprotective after 4 wk of subtotal nephrectomy. These studies may suggest that the uremic heart can still be protected by conditioning techniques. However, an experimental model of 4 wk of uremia may not properly reflect the clinical situation, since uremia remains unexplored until late stages of kidney disease in a significant number of patients (8). Thus the effect of prolonged experimental uremia on the cardioprotective effect elicited by endogenous cardioprotective mechanisms is still not known (8); however, the duration of the uremic condition may be an important determinant of the efficacy of ischemic pre- and postconditioning (8). Indeed, it has been shown earlier that 2 wk (54) of experimental diabetes protected the myocardium against ischemia/reperfusion injury; however, 4 or 8 wk of experimental diabetes abolished the protection by ischemic preconditioning (15, 54).

Therefore, in the present study we examined the influence of prolonged uremia (30 wk) on the severity of ischemia/reperfusion injury and the infarct size limiting effect of ischemic preconditioning.

MATERIALS AND METHODS

This investigation conforms to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication

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No. 85-23, Revised 1996) and was approved by the Animal Research Ethics Committee of the University of Szeged.

Adult male Wistar rats were used in the study. Animals were housed in pairs in individually ventilated cages (Sealsafe IVC system, Italy) and were maintained in a temperature-controlled room with a 12-h:12-h light/dark cycles throughout the study. Standard rat chow and tap water were supplied ad libitum. Drinking water contained 1 mg/100 g body wt iron (II) sulfate to attenuate the development of severe anemia.

Experimental setup. Experimental prolonged uremia was induced by partial (5/6) nephrectomy. Animals underwent sham operation or partial nephrectomy in two phases (Fig. 1) (42). There was no difference in mortality between sham-operated and partially nephrectomized groups. At *week 29*, cardiac function was assessed by transthoracic echocardiographic examination. At *week 29*, a group of animals were placed for 24 h in metabolic cages to estimate creatinine clearance and to measure urine creatinine and protein levels. At *week 30*, rats were anesthetized, and hearts were isolated and perfused ex vivo by oxygenated Krebs-Henseleit solution according to Langendorff as described previously (31). Immediately after excision of the heart, blood was collected from the thoracic cavity to measure plasma uric acid, carbamide, and creatinine levels to verify the development of uremia. Some plasma was used for the determination of ANG II as an indirect marker of hypertension and hypertrophy (20, 27, 28, 43, 58) and nitrotyrosine as a marker of systemic nitrosative stress (7). To assess the cardioprotective effect of ischemic preconditioning in the hearts of uremic and sham-operated animals, the perfused hearts were subjected to ischemia/reperfusion with or without preconditioning protocol. At the end of the appropriate perfusion protocol, the coronary artery was reoccluded and the area at risk and the infarcted area were delineated using an Evans blue/triphenyltetrazolium chloride double staining method (6).

Partial nephrectomy. Anesthesia was induced by intraperitoneal injection of pentobarbital sodium (Euthasol; 50 mg/kg; Produlab Pharma b.v., Raamsdonksveer, The Netherlands). After depilation and a ventral midline incision in the abdominal wall, the intestines were retracted laterally to expose the animal's left kidney. The kidney was freed from the perirenal adipose tissue and renal capsule. Two pieces of sutures (5-0 Mersilk; Ethicon, Sommerville, NJ) were placed around both poles of the kidney approximately at their 1/3 position. The sutures were gently ligated around the kidney. The 1/3 kidney on both ends was excised right beyond the ligatures. The abdominal incision was closed with running sutures. One week after the first operation, animals were anesthetized and prepared as described above (42). The right kidney was freed from the surrounding adipose tissue and renal capsule, and then it was pulled out of the incision gently. The adrenal gland, which is attached loosely to the anterior pole of the kidney, was gently freed and was placed back into the abdominal cavity. The renal blood vessels and the ureter were ligated, and the right kidney was removed (29). The incision was then closed with running sutures, and povidone iodide was applied on the surface of the skin. After operation, animals were placed on a warm heating pad until they become conscious again. As a post-operative medication, 0.3 mg/kg nalbuphine hydrochloride (Nalbuphine 10 mg/ml; TEVA, Debrecen, Hungary) was administered subcutaneously. Antibiotics (Enroxil, 75 mg; Krka, Slovenia) and analgesics (10 mg/l of nalbuphine hydrochloride, Nalbuphine; TEVA) were administered in tap water for 2 days after both surgeries.

Transthoracic echocardiography. Cardiac function was assessed by transthoracic echocardiographic examination 29 wk after the second surgery. Echocardiography was performed as described previously (39). The rats were anesthetized with pentobarbital sodium (Euthasol, 40 mg/kg body wt ip), the chest was shaved, and the animal was placed in supine position onto a heating pad. Two-dimensional, M-mode and Doppler echocardiographic examinations were performed in accordance with the criteria of the American Society of Echocardiography with a Vivid 7 Dimension ultrasound system (General Electric Medical Systems) using a phased array 5.5–12 MHz transducer (10S probe). Data of three consecutive heart cycles were analyzed (EchoPac Dimension software; General Electric Medical Systems) by an experienced investigator in a blinded manner. The mean values of three measurements were calculated and used for

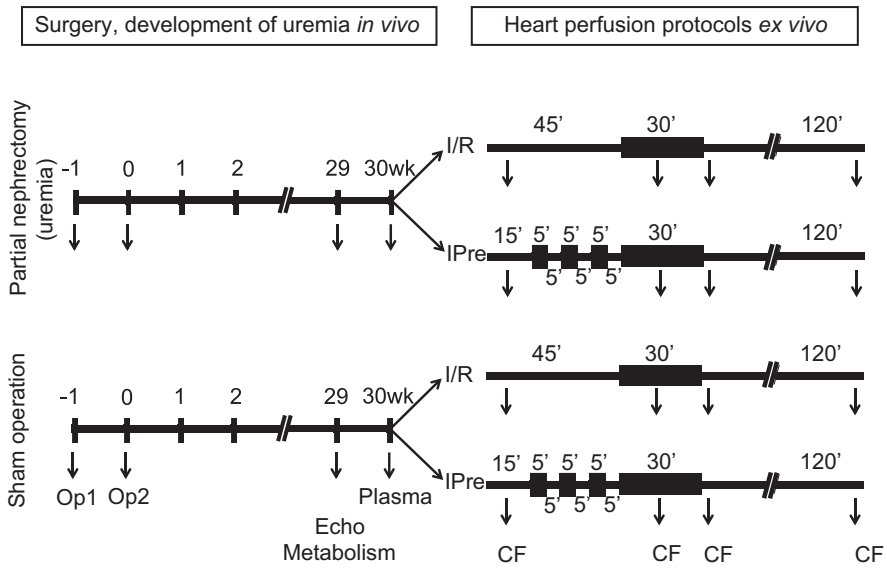

Fig. 1. Wistar rats underwent sham operation or partial nephrectomy in 2 phases. First, 2/3 of the left kidney was ligated and excised (Op1). One week later, the right kidney was removed (Op2). Corresponding time-matched sham operations were performed in the sham group. At *week 29*, cardiac function was assessed by transthoracic echocardiographic examination (Echo). In this week the animals (12–12 in uremia and sham groups) were placed for 24 h in a metabolic cage (Metabolism) to determine urine creatinine and protein levels. At *week 30*, rats were anesthetized and blood was collected from thoracic cavity to measure plasma carbamide and creatinine levels (Plasma). Hearts were then isolated and perfused according to Langendorff. After 15 min of aerobic perfusion, hearts were subjected to either preconditioning induced by 3 intermittent periods of 5-min ischemia/reperfusion (I/R) cycles or a time-matched (30 min) aerobic perfusion, both followed by a 30-min coronary occlusion and 2-h reperfusion (n = 6–9 in each group). Coronary flow (CF) was measured 10 min after the start of perfusion, in the 15th min of ischemia, in the first 5 min of reperfusion and at the end of the reperfusion. IPre, ischemic preconditioning (IPre).

statistical evaluation. Systolic and diastolic wall thickness parameters were obtained from parasternal short-axis view at the level of the papillary muscles and long-axis view at the level of the mitral valve. The left ventricle diameters were measured by means of M-mode echocardiography from long-axis and short-axis views between the endocardial borders. Functional parameters including left ventricular end-diastolic volume, left ventricular end-systolic volume, and ejection fraction were calculated on four-chamber view images. Diastolic function was assessed using pulse-wave Doppler across the mitral valve from the apical four-chamber view. Early (E) and atrial (A) flow velocity as well as mitral valve deceleration time and isovolumic relaxation time provide an indication of diastolic function.

Urine creatinine and total protein levels. At *week 29*, the animals were placed in a metabolic cage (Techniplast, Italy) for 24 h to collect urine for the measurement of urine creatinine and protein levels to verify the development of advanced uremia.

Urine creatinine and urine protein levels were measured by standard laboratory methods as described previously by others (11, 40).

Plasma carbamide and creatinine levels. Blood was collected from the thoracic cavity after isolation of the heart to measure plasma carbamide and creatinine levels to verify the development of chronic uremia at *week 30*. Plasma carbamide and creatinine levels were measured in triplicate, using commercially available colorimetric assay kits (Diagnosticum, Budapest, Hungary) applying enzymatic determinations adapted to 96-well plates.

Creatinine clearance. Creatinine clearance, an indicator of renal function, was calculated according to the standard formula (urine creatinine concentration [μM] × urine volume for 24 h [ml])/(plasma creatinine concentration [μM] × 24 × 60 min).

Hematocrit and hemoglobin level. Hematocrit and hemoglobin were measured from whole blood by means of a blood gas analyzer (Radiometer ABL 77; Radiometer Medical, Bronshoj, Denmark) at *week 30* to verify the development of renal anemia.

Ex vivo cardiac perfusions and infarct size determination. At *week 30*, rats were anesthetized and hearts were isolated and perfused at 37°C according to Langendorff with oxygenated Krebs-Henseleit buffer as previously described (14, 31). Hearts from the sham-operated and the uremic groups were further subdivided into two subgroups (n = 6–9) and subjected to either a nonconditioning or a preconditioning perfusion protocol, respectively (Fig. 1). Nonconditioned hearts were subjected to time-matched aerobic perfusion followed by test ischemia-reperfusion induced by a 30-min occlusion of the left descending coronary artery. Preconditioned hearts were subjected to three intermittent periods of 5-min ischemia/reperfusion followed by test ischemia/reperfusion. A 3-0 silk suture was placed around the origin of the left descending coronary artery and passed through a plastic tube to form a snare. After stabilization of the heart, coronary occlusion was induced by pulling the ends of the suture taut and clamping the snare onto the epicardial surface. Reperfusion was achieved by releasing the snare as previously described (9, 31). Heart rate and coronary flow were monitored throughout the perfusion protocol (Fig. 1). At the end of the 2-h reperfusion protocol, the coronary artery was reoccluded and 5 ml of 0.1% Evans blue dye (Merk, Germany) was injected into the aorta to delineate the area-at-risk zone. Stained hearts were weighed, frozen, sliced, and incubated at 37°C in 1% triphenyl-tetrazolium chloride (Sigma Aldrich, Germany) to delineate infarcted tissue. Slices were then fixed and quantified by planimetry using Infarctsize 2.5 software (Pharmahungary, Szeged, Hungary) (31). Infarct size was expressed as a percentage of the area-at-risk zone (6). The area at risk was calculated as a percentage of total ventricular area (6).

Plasma ANG II level. Plasma ANG II level was determined as a marker of hypertension and left ventricular hypertrophy. The level of ANG II in blood plasma of uremic and sham-operated rats was determined with enzyme linked immunosorbent assay (ELISA) kits recognizing rat peptides (Phoenix Pharmaceuticals) in accordance with the manufacturer's instructions.

Plasma 3-nitrotyrosine level. Plasma 3-nitrotyrosine level was determined as a marker of systemic nitrosative stress. Free 3-nitrotyrosine level was measured by ELISA (Cayman Chemical) as described earlier (7) from sham and uremic plasma samples taken at *week 30*. Briefly, supernatants of deproteinized plasma samples were concentrated and then incubated overnight with anti-nitrotyrosine rabbit IgG specific to free 3-nitrotyrosine and nitrotyrosine acetylcholinesterase tracer in precoated (mouse anti-rabbit IgG) microplates followed by development with Ellman's reagent. Free nitrotyrosine content was expressed as nanograms per milliliter plasma.

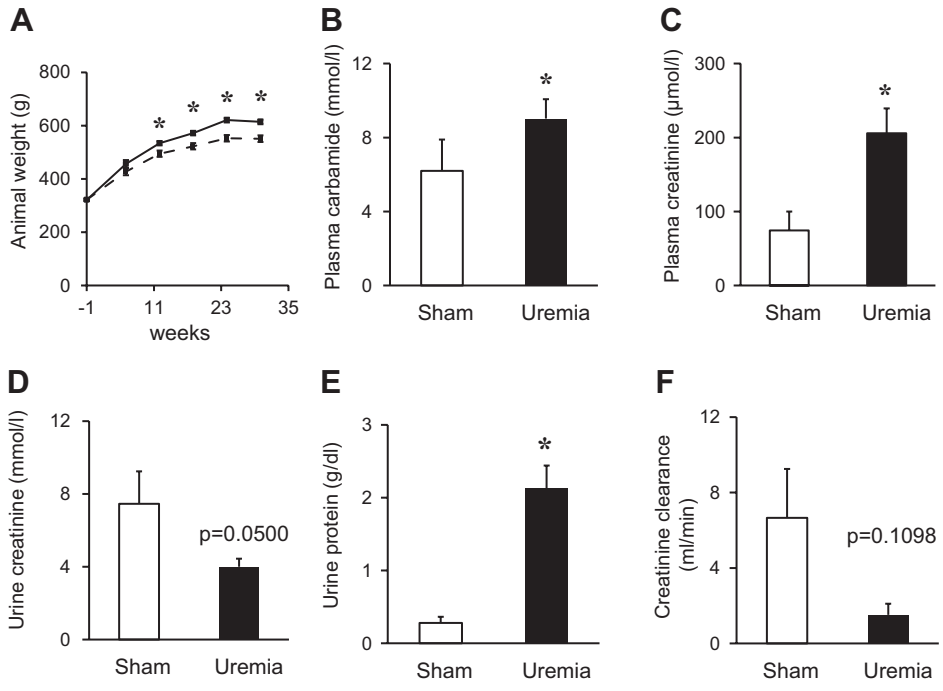

Fig. 2. Animal weight (*A*) shown in every 6 wk. Solid line, Sham; dashed line, uremia. Plasma carbamide (*B*; n = 14 in both groups) and creatinine (*C*, n = 12–15), urine creatinine (*D*; n = 12 in each groups) and protein concentrations (*E*; n = 10–12) and creatinine clearance (*F*; n = 9 to 10) in both sham-operated and uremic rats are shown. Values are means ± SE. *$P < 0.05$.

Plasma uric acid level. Plasma uric acid level was measured to further characterize our uremic model. Plasma uric acid level was measured in duplicate, using a commercially available colorimetric assay kit (Diagnosticum, Budapest, Hungary) according to the manufacturer's instructions.

Statistical analysis. All values are presented as means ± SE. Two-way ANOVA was used to determine the effect of uremia or preconditioning on infarct size, area at risk, and coronary flow. To analyze the effect of preconditioning on infarct size within sham-operated as well as uremic groups, an unpaired *t*-test was then applied. All other parameters were analyzed by unpaired *t*-tests comparing data in the uremic groups with sham-operated controls. $P < 0.05$ was accepted as a statistically significant difference.

RESULTS

Characterization of prolonged uremia. To verify the development of long-term uremia induced by partial nephrectomy, body weight was monitored during the experiment and concentrations of several plasma and urine metabolites were measured at *week 29*. Partially nephrectomized rats showed significantly lower body weights starting from *week 7* showing uremic cachexia (Fig. 2*A*). Plasma carbamide and creatinine levels were markedly increased in partially nephrectomized rats representing the uremic state of these animals (Fig. 2, *B* and *C*). Plasma glucose levels were similar in both the sham-operated and the partially nephrectomized groups (5.7 ± 0.1 vs. 5.0 ± 0.2; $n = 13$–16; not significant). Urine protein concentration was significantly increased in the partially nephrectomized rats as compared with sham-operated controls (Fig. 2*E*) showing an impaired renal function. Moreover, urine creatinine level and creatinine clearance showed a marked but statistically not significant decrease at the level of $P < 0.05$ in partially nephrectomized rats (Fig. 2, *D* and *F*). Hematocrit and hemoglobin levels were significantly decreased in uremic animals (44.7 ± 2.2% and 15.0 ± 0.7 g/dL, respectively; $n = 6$) when compared with sham-operated rats (54.2 ± 1.0% vs. and 17.7 ± 0.3 g/dL respectively; $n = 10$) showing renal anemia.

Effect of prolonged uremia on myocardial morphology and function. Transthoracic echocardiography was performed at *week 29* to investigate whether the development of prolonged uremia leads to alteration of myocardial morphology and function. Left

Table 1. *Effects of uremia on various in vivo left ventricular morphological and functional parameters measured by transthoracic echocardiography*

	Echocardiography			
	View/Mode	Sham	Uremia	*P* Value
Morphology				
n		14	16	
Wall thickness, mm				
Anterior				
Systolic	short axis/MM	3.31 ± 0.05	3.59 ± 0.11*	0.032
Diastolic	short axis/MM	1.87 ± 0.07	1.97 ± 0.09	0.371
Lateral				
Systolic	short axis/MM	3.47 ± 0.11	3.38 ± 0.18	0.796
Diastolic	short axis/MM	2.14 ± 0.40	1.98 ± 0.08	0.270
Posterior				
Systolic	long axis/MM	3.77 ± 0.11	3.81 ± 0.11	0.781
Diastolic	long axis/MM	2.18 ± 0.07	2.30 ± 0.08	0.287
Septal				
Systolic	long axis/MM	2.70 ± 0.12	3.04 ± 0.13*	0.046
Diastolic	long axis/MM	2.25 ± 0.10	2.26 ± 0.08	0.961
Left ventricular end-diastolic diameter, mm	long axis/MM	7.25 ± 0.17	6.95 ± 0.14	0.176
Left ventricular end-diastolic diameter, mm	short axis/MM	7.23 ± 0.18	7.23 ± 0.19	0.967
Left ventricular end-systolic diameter, mm	long axis/MM	3.82 ± 0.16	3.55 ± 0.15	0.237
Left ventricular end-systolic diameter, mm	short axis/MM	3.77 ± 0.20	3.53 ± 0.19	0.400
Systolic function				
n		14	16	
Ejection fraction, %	four chamber/2D	54.9 ± 1.7	48.9 ± 1.4*	0.012
Stroke volume, μl	four chamber/2D	252.2 ± 13.8	222.9 ± 10.1	0.093
Left ventricular volume, μl				
End systolic	four chamber/2D	219.3 ± 23.1	240.0 ± 18.7	0.487
End diastolic	four chamber/2D	477.0 ± 36.1	461.7 ± 27.3	0.736
Heart rate, beats/min	four chamber/2D	391.1 ± 6.9	396.1 ± 4.9	0.565
Diastolic function - Doppler imaging				
n		13	14	
E-wave, m/s	four chamber/PW	0.82 ± 0.03	0.85 ± 0.03	0.664
A-wave, m/s	four chamber/PW	0.50 ± 0.03	0.51 ± 0.02	0.889
E-to-A ratio	four chamber/PW	1.69 ± 0.07	1.72 ± 0.09	0.830
Deceleration time, ms	four chamber/PW	33.98 ± 1.95	34.39 ± 1.75	0.885
Isovolumic relaxation time, ms	four chamber/PW	25.05 ± 0.89	21.69 ± 0.9*	0.025
Left ventricular gradient, mmHg				
Maximal	four chamber/PW	2.89 ± 0.25	3.09 ± 0.24	0.598
Mean	four chamber/PW	1.31 ± 0.10	1.47 ± 0.12	0.351

Values are means ± SE; $n = 13$–16. Transthoracic echocardiographic measurements were performed 29 wk after sham operation or partial nephrectomy. *$P < 0.05$ vs. sham, unpaired *t*-test. MM, M (motion) mode; 2D, 2-dimensional; PW, pulse wave; E-wave, early ventricular filling velocity; A-wave, late or atrial ventricular filling velocity.

Table 2. *Effects of uremia on ex vivo coronary flow data*

Coronary flow			
Time points of the measurements	Sham	Uremia	*P* Value
During nonconditioning perfusion protocol			
n	8	9	
Baseline, ml/min	16.9 ± 1.3	13.1 ± 1.1*	0.044
15th min of ischemia, ml/min	8.6 ± 0.9	7.4 ± 0.5	0.260
First 5 min of reperfusion, ml/min	19.0 ± 1.3	16.9 ± 1.6	0.367
End of reperfusion, ml/min	13.0 ± 0.7	9.4 ± 1.1*	0.023
During preconditioning perfusion protocol			
n	6	7	
Baseline, ml/min	17.5 ± 1.1	13.0 ± 0.6*	0.007
15th min of ischemia, ml/min	8.6 ± 0.7	6.4 ± 0.7	0.071
First 5 min of reperfusion, ml/min	22.2 ± 1.1	15.5 ± 1.7*	0.016
End of reperfusion, ml/min	14.1 ± 0.9	8.4 ± 0.8*	0.003

Values are means ± SE; $n = 6$–9. Studies were performed on ex vivo Langendorff heart perfusion system 30 wk after sham operation or partial nephrectomy, *$P < 0.05$ vs. sham, unpaired *t*-test.

ventricular systolic anterior and septal wall thicknesses were increased in uremic rats as compared with sham-operated controls (Table 1). However, there was no difference in diastolic anterior and septal wall thickness between the uremic and sham-operated group. In addition, left ventricular lateral and posterior wall thickness both in systole and diastole were similar in the uremic and the sham-operated groups (Table 1). Uremic animals demonstrated a mild reduction both in left ventricular systolic and diastolic function at *week 29*. Ejection fraction was significantly reduced in the uremic group as compared with the sham-operated control group (Table 1). Stroke volume showed a tendency of decrease in uremic animals as compared with sham-operated controls (Table 1). Heart rate and left ventricular end-systolic and end-diastolic volumes were similar in both uremic and sham-operated groups (Table 1). Isovolumic relaxation time was decreased in uremic rats as compared with sham-operated controls (Table 1). Other diastolic functional parameters including early and late ventricular filling velocity, E/A ratio, deceleration time, and maximal and mean left ventricular gradient were not changed in the uremic group as compared with sham-operated controls (Table 1).

Effect of prolonged uremia on ex vivo morphological and functional parameters. Coronary flow and morphological parameters including heart weight, left kidney weight, and tibia length were measured at *week 30* to investigate whether advanced uremia influences ex vivo functional and morphological parameters of the heart. Coronary flow was significantly reduced in uremic groups when compared with corresponding sham-operated control groups as assessed by two-way ANOVA (Table 2). The ratio of heart weight to body weight showed a tendency of increase in uremic animals (Table 3), as a result of a significantly lower body weight in the uremic group (Fig. 2*A*). However, there was no difference in heart weight and heart weight-to-tibia length ratio between the uremic and the sham-operated control group (Table 3). In addition, the weight of the whole left kidney in the sham-operated group and the remaining one-third of the left kidney in the uremic group were similar, suggesting a marked renal hypertrophy in the uremic animals (Table 3).

Effect of ischemic preconditioning on infarct size. Infarct size was measured at *week 30* to investigate the severity of ischemia/reperfusion injury and the cardioprotective effect of ischemic preconditioning in prolonged uremia (Fig. 3*A*). Preconditioning significantly decreased infarct size; however, the presence of prolonged uremia did not significantly influence the size of infarction as assessed by two-way ANOVA. Additional analysis with unpaired *t*-tests showed a significant infarct size limiting effect of preconditioning in hearts of both uremic and sham-operated rats (Fig. 4*B*). The area-at-risk zone was not affected significantly in any of the groups (Fig. 4*A*).

Effect of prolonged uremia on plasma ANG II, 3-nitrotyrosine, and uric acid levels. Partially nephrectomized rats showed significantly higher plasma ANG II level (Fig. 4*A*), which is a well-known marker of hypertension and left ventricular hypertrophy.

Plasma 3-nitrotyrosine level was determined as a marker of peroxynitrite and systemic nitrosative stress. Plasma 3-nitrotyrosine level was markedly increased in uremic rats as compared with sham-operated controls, representing increased systemic nitrosative stress in uremic animals (Fig. 4*B*).

Plasma level of uric acid, a well-known antioxidant, was significantly increased in the partially nephrectomized rats as compared with sham-operated controls (Fig. 4*C*) showing increased antioxidant capacity in uremic rats.

DISCUSSION

We have found here that although 30 wk of experimental renal failure leads to severe metabolic changes and the development of myocardial dysfunction, the cardioprotective effect of ischemic preconditioning is still observed. This is the first demonstration that prolonged uremia does not interfere with the cardioprotective effect of ischemic preconditioning.

The cardioprotective effect of ischemic pre- and postconditioning is inhibited by several comorbid conditions and risk factors such as aging (3, 47, 59), hyperlipidemia (14–17, 23, 36, 55), and diabetes (35, 48, 54). However, recent evidence suggests that the cardioprotective effect of pre- and postconditioning is maintained in subacute renal failure (4 wk) (4) and short-term (10 wk) (32) model of uremia. In a preliminary study, we have demonstrated for the first time in the literature that the infarct size limiting effect of postconditioning was still present 10 wk after subtotal nephrectomy, resulting in uremia in rats (32). Moreover, an extensive study recently published by Byrne et al. (4) has reported that ischemic preconditioning, remote ischemic conditioning, and ischemic postconditioning are still cardioprotective after 4 wk of subtotal nephrectomy. In addition, in the same study ischemic preconditioning was

Table 3. *Effect of uremia on various ex vivo morphological parameters*

Measurement	Sham	Uremia	*P* Value
n	14	12	
Heart weight, g	2.10 ± 0.09	2.10 ± 0.10	0.980
Heart weight/body weight × 10^3	3.40 ± 0.17	3.77 ± 0.15	0.126
Heart weight/tibia length × 10^2	4.00 ± 0.20	4.20 ± 0.20	0.483
Left kidney weight, g	Full kidney weight 1.90 ± 0.11	1/3 kidney weight 2.04 ± 0.09	—

Values are means ± SE; $n = 12$–14, unpaired *t*-test. Measurements were performed 30 wk after sham operation or partial nephrectomy.

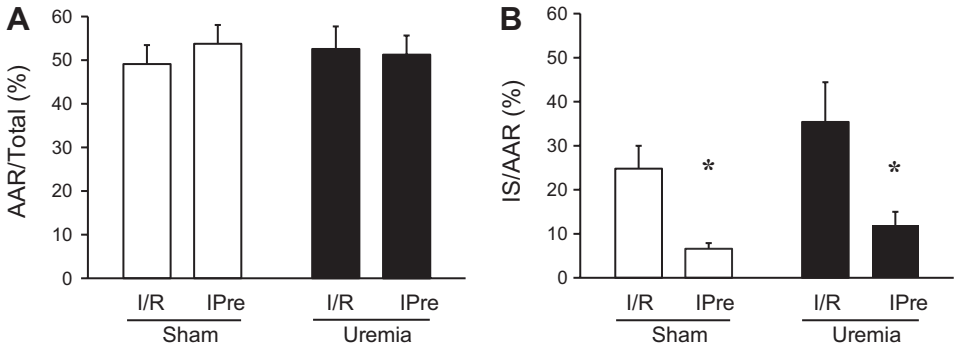

Fig. 3. Area at risk (AAR; *A*) and infarct size (IS; *B*) after ischemia/reperfusion (I/R) or ischemic preconditioning (IPre) in both sham-operated and uremic groups. Values are means ± SE; $n = 6–9$ in each group. Two-way ANOVA on all groups showed that preconditioning significantly decreased infarct size; however, uremia did not influence infarct size. *Significant difference (*$P < 0.05$) by an unpaired *t*-test between I/R and IPre groups.

shown to limit infarct size in an adenine-enriched diet-induced model of uremia in rats (4). Although the uremic state seems to be too short and was not characterized thoroughly in Byrne's aforementioned studies (4, 8), they may suggest that the uremic heart can be still protected by conditioning techniques. Indeed, an experimental model of 4 wk of uremia probably may not properly reflect the clinical situation, since uremia may remain unexplored for a long time in patients (8, 37). Additionally, it has been shown that 5/6 partial nephrectomy followed by a postoperative duration of 3 wk does not lead to advanced uremia in rats (10).

Therefore, in the present study we used a prolonged (30 wk) postoperative duration following partial nephrectomy to induce a more severe long-term uremic condition for investigating the cardioprotective effect of preconditioning. In this model, here we have found characteristic biochemical and pathophysiological signs of prolonged uremia, including cachexia, increased plasma uric acid, carbamide and creatinine levels, decreased hematocrit and hemoglobin, increased urine protein, and decreased creatinine concentration, as well as diminished creatinine clearance. In patients with end-stage renal failure, uremic cardiomyopathy is a common complication and reported to be a prognostic factor of cardiovascular mortality (24, 50, 53). In the present study, we have found increased septal and anterior wall thickness in uremic animals. Additionally, plasma ANG II level, which is an indirect marker of hypertension and left ventricular hypertrophy (20, 27, 28, 43, 58), was also higher in uremic rats. These results together with literature data (1, 33, 38, 41) suggest the development of a minimal left ventricular hypertrophy in uremic animals at *week 30* in our present study. In this model, we have found here that ischemic preconditioning is still effective in prolonged uremic condition. This is a refreshingly interesting result in the light of the fact that several metabolic diseases including diabetes and hyperlipidemia inhibit the endogenous cardioprotective mechanisms of ischemic conditioning.

The reason why the complex metabolic changes of uremia, which significantly affect several intracellular signaling pathways in the heart and lead to myocardial dysfunction, do not affect the overall efficacy of cardioprotection by ischemic preconditioning is unknown. It has been previously shown that 6- to 8-wk-long chronic renal failure leads to increased oxidative and nitrosative stress in the rat heart (1, 51, 56, 57). Here we have also shown increased nitrosative stress in a 30-wk model of prolonged uremia. It is well known that the cardioprotective effect of ischemic preconditioning is lost in metabolic diseases associated with increased myocardial oxidative and nitrosative stress (15). Therefore, our present findings showing that the cardioprotective effect of ischemic preconditioning is surprisingly still preserved in uremic animals despite the increased systemic nitrosative stress are particularly exciting. These results suggest that even though prolonged uremia leads to some deleterious effects (e.g., mild cardiac dysfunction, tendency of increased infarct size), it likely induces some complex compensatory alterations supporting maintained cardioprotection by ischemic preconditioning. This hypothesis is further supported by our present finding showing elevated plasma levels of uric acid, a known antioxidant, in prolonged uremia. However, further studies should be carried out to determine the precise role of oxidative/nitrosative stress in the mechanism of preconditioning in uremia.

Upregulation of renal renin-angiotensin-aldosterone system was also shown in rats with partial nephrectomy in our present study similarly to findings of other research groups (25, 33, 41). In our present study, increased plasma ANG II levels together with echocardiographic data suggest the development of a minimal left ventricular hypertrophy at *week 30* in uremic animals. In this aspect our study is in line with other studies

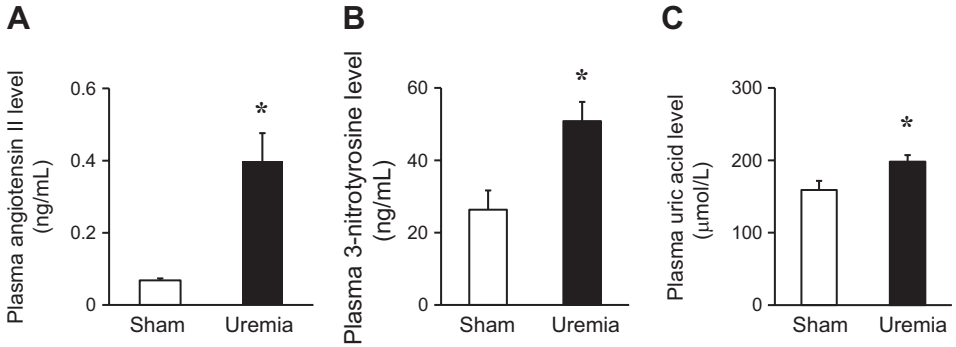

Fig. 4. Plasma ANG II (*A*; $n = 9$ to 10), nitrotyrosine (*B*; $n = 9$ to 10), and uric acid (*C*; $n = 9$ to 10) levels in both sham-operated and uremic rats. Values are means ± SE. *$P < 0.05$.

showing that ischemic preconditioning is still cardioprotective in case of left ventricular hypertrophy (45, 52) and hypertension (12, 13, 52) associated with increased plasma ANG II level (15). Therefore, we believe that the presence of a minimal cardiac hypertrophy and mild left ventricular dysfunction in uremic animals might not interfere with the cardioprotective mechanism of ischemic preconditioning. However, it cannot be excluded that the cardioprotection by preconditioning may be lost with the progression of the disease and development of severe heart failure in end-stage renal failure, as it has been shown that endogenous cardioprotection is lost in severe heart failure (22, 30, 34). In addition, renal failure seems to interact with some protein kinases (33, 38) suggested to be involved in the mechanism of ischemic preconditioning. Taken together, the overall net changes in uremia seem to preserve the cardioprotective effect of ischemic preconditioning; however, the exact mechanisms remain to be elucidated in further studies.

In conclusion, our present study suggests that patients suffering from long-term uremia may also benefit from cardioprotection by ischemic preconditioning. This is particularly important since acute myocardial infarction frequently occurs in patients with late stages of renal failure. Because uremic patients are regularly excluded from clinical trials, there is a need for clinical studies to investigate the cardioprotective effect of conditioning techniques in patients with chronic renal failure suffering from acute myocardial infarction.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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