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Effects of exercise training on nitric oxide synthase in the kidney of spontaneously hypertensive rats

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SUMMARY

1. Exercise training is known to have antihypertensive effects in humans and animals with hypertension, as well as to exhibit renal protective effects in animal models of hypertension and chronic renal failure. However, the mechanisms regulating these effects of exercise training remain unclear.

2. The present study examined the effects of exercise training on nitric oxide synthase (NOS) in the kidneys of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats.

3. Male SHR and WKY rats were randomly divided into a sedentary group and a treadmill exercise group for 8 weeks. Systolic blood pressure (SBP) was measured every 2 weeks by the tail-cuff method and urine and blood samples were collected after the exercise protocol. Nitric oxide synthase activity and protein expression and endothelial (e) NOS phosphorylation in the kidney were examined.

4. Exercise training significantly lowered SBP, decreased urinary albumin excretion, thiobarbituric acid-reactive substances levels and renal NADPH oxidase activity, and increased creatinine clearance in SHR. Exercise training significantly increased plasma and urinary nitrate/nitrite, NOS activity and eNOS and neuronal NOS expression, but decreased eNOS phosphorylation at Ser^{1177} and Thr^{495} in kidneys of SHR and WKY rats.

5. Renal NOS may be involved in the antihypertensive and renal protective effects of exercise training in SHR.

Key words: exercise, hypertension, kidney, nitric oxide synthase, spontaneously hypertensive rats..

INTRODUCTION

Numerous epidemiological studies have reported that exercise has regulatory effects on blood pressure.[1] In experimental studies, exercise has also been reported to reduce blood pressure in spontaneously hypertensive rats (SHR),[2] Dahl salt-sensitive rats[3] and angiotensin II-infused rats.[4] In addition to its blood pressure regulatory effects, exercise has renal protective effects, including decreasing plasma creatinine and proteinuria and improving glomerular sclerosis, in rats with chronic renal failure (CRF)[5,6] or diabetic nephropathy,[7] as well as in fructose-fed SHR.[8] However, the mechanisms responsible for the beneficial effects of exercise are not fully understood.

In response to exercise, increased blood flow can cause shear stress, which leads to endothelium-derived nitric oxide (NO) production in the heart, aorta and skeletal muscle.[9,10] NO is a vasodilatory factor synthesised by the three isoforms of NO synthase (NOS), namely endothelial (e) NOS, neuronal (n) NOS and inducible (i) NOS, that has an important role in blood pressure control. For example, NOS inhibition increases blood pressure in SHR and normotensive Wistar–Kyoto (WKY) rats.[11] However, administration of L-arginine, a substrate for NOS, lowers blood pressure in Dahl salt-sensitive rats.[12] Furthermore, eNOS-knockout mice and transgenic mice exhibit hypertension and hypotension, respectively,[13,14] whereas delivery of the human eNOS gene lowers blood pressure in SHR.[15]

In addition, NO has various renal effects, including regulation of renal haemodynamics and inhibition of renin secretion, tubular Na^+ reabsorption, tubuloglomerular feedback (TGF) and sympathetic nerve activity.[16,17] These renal actions of NO are involved in its antihypertensive and renal protective effects.[16,17] Chronic NOS inhibition also induces renal damage, proteinuria and glomerular sclerosis,[11,18] whereas eNOS and nNOS protein expression is downregulated in the kidneys of rats with CRF.[19,20]

Although renal NO is crucial for the regulation of renal function and blood pressure, the effects of exercise on renal NO have not been fully clarified. In contrast with the effects of exercise on blood flow in the heart, aorta and skeletal muscle,[9,10] renal blood flow decreases during exercise[21] and a previous study reported that renal eNOS expression and NOS activity were reduced after acute exercise.[22] The downregulation of renal NOS by acute exercise contrasts with the antihypertensive and renal protective effects of exercise. Therefore, acute and chronic exercise may have different effects on renal NOS.

The localization and involvement of renal NOS in the renal vessels and tubules have been studied extensively. The renal vasa recta, glomeruli and afferent arterioles contain large amounts of constitutive NOS isoforms (eNOS and nNOS),[23] whereas nNOS in the macula densa of the cortex contributes to the regulation of

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TGF and renin secretion.[17,24] A recent study suggested that adenosine A_{2A} receptor-mediated attenuation of TGF was linked to stimulation of eNOS in the afferent arteriole.[25] Furthermore, NO has been reported to contribute to improvements in medullary blood flow in Dahl salt-sensitive rats,[26] suggesting that NO and NOS play different roles in each segment of the kidney. Thus, in the present study, we hypothesized that chronic exercise would cause antihypertensive effects with upregulation of NOS in the kidney and cardiovascular system. In particular, we focused on the renal effects of exercise and examined changes in NOS in various renal sections (i.e. cortex, outer medulla and inner medulla) of kidneys from SHR and WKY rats.

METHODS

Animals

Five-week-old male SHR/Izm and WKY/Izm rats (n = 12 per strain; 110–130 g) were obtained from SLC (Shizuoka, Japan). Rats were housed in the animal care facility at Tohoku University Graduate School of Medicine under controlled temperature (24°C) and a 12 h light–dark cycle. All rats had free access to standard laboratory chow and water. All experiments were approved by the Animal Welfare Committee of Tohoku University School of Medicine.

Experimental groups and exercise training protocol

Spontaneously hypertensive and WKY rats were each randomized into a sedentary (Sed) or exercise (Ex) group (n = 6 per group; Sed-WKY, Ex-WKY, Sed-SHR and Ex-SHR). Rats in the exercise group underwent forced treadmill exercise, with an electrode positioned behind the treadmill (KN-73; Natsume Industries, Tokyo, Japan), for 8 weeks (20 m/min, 60 min/day, 6 days/week). Rats were exercised for 10 min/day at an initial treadmill speed of 10 m/min up a 0% grade. The speed of the treadmill was increased gradually to 20 m/min and the duration of exercise training was increased to 60 min/day for 1 week. Oxygen consumption (V_{O_2}) when rats were running at a speed of 20 m/min corresponded to approximately 50–60% of the peak V_{O_2}. This is a low-intensity load that effectively reduces sympathetic tone and blood pressure in SHR.[27]

Blood pressure measurements

Systolic blood pressure (SBP) was measured every 2 weeks in conscious rats using an indirect tail-cuff method (Model UR-5000; Ueda, Tokyo, Japan), as described previously.[28] Before the first measurement, SBP was measured more than five times per day for 1 week so that the rats became accustomed to the apparatus. Measurements were performed by an observer blinded to the treatment group and were repeated more than five times until the rats became quiet and a stable SBP measurement was obtained.

Plasma and urinary parameters and measurements of nitrate/nitrite

After the exercise protocol, all rats were housed in individual metabolic cages (Model ST; Sugiyama-General, Tokyo, Japan) for 3 days to acclimatize to the conditions. Urine samples were collected on ice over a period of 24 h. On the final experimental day, all rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and blood samples collected after decapitation. The blood samples were centrifuged for 5 min at 1500 g and the supernatant collected and stored at −80°C. Total cholesterol, triglyceride, free fatty acids, glucose, blood urea nitrogen (BUN), creatinine and noradrenaline levels in the plasma and urinary sodium, creatinine and noradrenaline levels were determined using standard auto-analysis techniques (BML, Tokyo, Japan). Plasma renin activity was measured by SRL (Tokyo, Japan). Urinary albumin concentrations were determined using commercially available assay kits (AKRAL-120; Shibayagi, Shibukawa, Japan), as described previously.[29]

Plasma and urinary nitrate/nitrite (NO_x) were measured spectrophotometrically by the Griess reagent method[30] using a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA), with results expressed as μmol/L for plasma NO_x concentrations and μmol/day for urinary NO_x excretion.

Determination of nitric oxide synthase activity

After rats had been decapitated, their kidneys and thoracic aorta were quickly removed. The kidney was hemisected and sectioned into the cortex, the inner stripe of the outer medulla and the inner medulla. These tissues were homogenized in 100 mmol/L potassium buffer (pH 7.25) containing 30% glycerol, 1 mmol/L dithiothreitol and 0.1 mmol/L phenylmethylsulphonyl fluoride. The samples were snap-frozen in liquid nitrogen and stored at −80°C. Protein concentrations in the samples were determined using the Bradford method[31] with bovine γ-globulin as the standard (Bio-Rad Laboratories, Hercules, CA, USA).

For determination of NOS activity, the *in vitro* formation of NO_x by each tissue was evaluated using a commercially available NOS activity assay kit (Oxford Biomedical Research, Rochester Hills, MI, USA). Tissue samples (50 μg protein) were incubated for 5 h at 37°C with L-arginine, NADPH and an NADPH-regenerating system containing $NADP^+$, glucose 6-phosphate and glucose 6-phosphate dehydrogenase in 200 μL of 50 mmol/L HEPES buffer (pH 7.4) with 0.5 mmol/L EDTA. After termination of the reaction by cooling on ice for 5 min, reconstituted nitrate reductase (10 mU) was added to the reaction mixture and samples were incubated for 20 min at room temperature. The reaction mixture was then centrifuged at 12 500 g for 5 min at 4°C and 100 μL of the supernatant was mixed with Griess reagent before absorbance was determined at 540 nm. Under these conditions, NO production determined by NO_x formation was linear with time and protein concentration. Data for NOS activity are expressed as μmol/h per g protein.

Thiobarbituric acid-reactive substances levels and NADPH oxidase activity

Plasma and urinary thiobarbituric acid-reactive substances (TBARS) were measured as an index of lipid peroxidation using a colorimetric assay kit (Cayman Chemical), as described previously.[32] Data are expressed as nmol malondialdehyde (MDA)/mL for plasma TBARS levels and nmol MDA/day for urinary TBARS. In the present study, NADPH oxidase activity was

measured as an index of O_2^- generation by the lucigenin-enhanced chemiluminescence method. Briefly, proteins from renal cortical and medullary samples (200 μg) were resuspended in 1 mL Krebs'–HEPES buffer (composition (in mmol/L): NaCl 119; HEPES 20; KCl 4.6; $CaCl_2$ 1.2; Na_2HPO_4 0.15; KH_4PO_4 0.4; $MgSO_4$ 1.0; $NaHCO_3$ 25; glucose 5.5).

Background chemiluminescence was recorded using a tube luminescencer (PSN AB-2200; ATTO, Tokyo, Japan) for 5 min after the addition of lucigenin (10 μmol/L; Sigma-Aldrich, St Louis, MO, USA). After the addition of NADPH (100 μmol/L), chemiluminescence was measured for an additional 5 min. The activity of NADPH oxidase was determined by subtracting background values from values obtained after the addition of NADPH and is expressed as c.p.m./g protein.

Western blot analysis

The expression and phosphorylation of NOS were examined by western blot analysis, as described previously.[33] Briefly, protein samples (50 μg) were separated by electrophoresis on a 5.8% sodium dodecyl sulphate polyacrylamide gel. Proteins were transferred electrophoretically to nitrocellulose membranes and membranes were blocked by immersion into TBST-20 buffer (10 mmol/L Tris–HCl, 150 mmol/L NaCl, 0.08% Tween 20 and 10% non-fat dry milk). The membranes were then incubated at room temperature for 2 h with primary antibodies raised against eNOS, nNOS, iNOS and phosphorylated (p-) eNOS (Ser^{1177}, Thr^{495}; BD Transduction Laboratories, San Diego, CA, USA). Membranes were rinsed with TBST-20 and incubated at room temperature for 1 h with a horseradish peroxidase-conjugated anti-mouse IgG secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washing in TBST-20, immunoblots were developed using an enhanced chemiluminescence kit (Super Signal; Thermo Fisher Scientific, Waltham, MA, USA).

Neuronal NOS is known to have several protein isoforms,[34,35] with nNOS-α and nNOS-β present in the rat kidney.[36] The relative intensities of bands at 140 kDa for eNOS and p-eNOS, at 155 kDa for nNOS-α, at 135 kDa for nNOS-β and at 130 kDa for iNOS were quantified using ImageJ (v1.40; National Institutes of Health, Bethesda, MD, USA). The intensities of the bands for each NOS protein were normalized against those for β-actin, used as an internal standard. The intensity of the bands in the Sed-WKY group was assigned a value of 1. Ratios of relative intensities of the bands for p-eNOS protein against total eNOS protein were calculated, with the ratio in the Sed-WKY assigned a value of 1.

Statistical analysis

Data are presented as the mean ± SEM. Comparisons among the four groups were performed using one-way ANOVA followed by Tukey's post hoc test. Two-tailed $P < 0.05$ was considered significant.

RESULTS

Effects of exercise on systolic blood pressure

The results for SBP are shown in Fig. 1. After 8 weeks, SBP was significantly lower in rats in the Ex-SHR group compared with those in the Sed-SHR group (213 ± 2 vs 224 ± 3 mmHg, respectively). In contrast, there were no significant differences between the Sed-WKY and Ex-WKY groups (155 ± 3 vs 155 ± 3 mmHg, respectively).

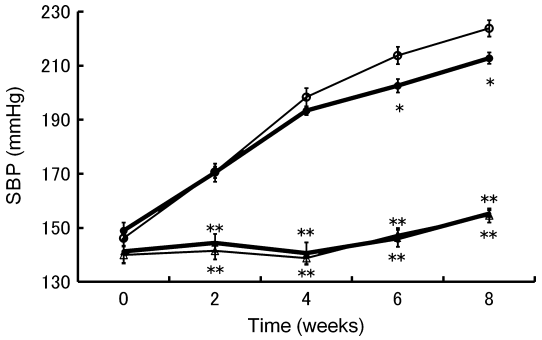

Fig. 1 Effects of exercise training on systolic blood pressure (SBP) in Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Wistar–Kyoto rats and SHR were randomized into sedentary (Sed-WKY, Sed-SHR) and exercised (Ex-WKY, Ex-SHR) groups. Exercised groups were given treadmill training for 8 weeks. (○), Sed-SHR; (●), Ex-SHR; (△), Sed-WKY; (▲), Ex-WKY. Data are the mean ± SEM ($n = 6$ rats per group). *$P < 0.05$, **$P < 0.01$ compared with the Sed-SHR group.

Effects of exercise on biochemical parameters

Table 1 summarizes the plasma and urinary parameters of the four groups at the end of the experiment. There were no significant differences in plasma levels of total cholesterol, triglycerides, free fatty acids, glucose, BUN and noradrenaline or in urinary sodium levels between exercised and sedentary rats for either strain, although exercise tended to decrease levels of plasma renin activity and urinary noradrenaline in both strains. Exercise significantly decreased plasma creatinine and increased creatinine clearance in SHR, but had no effect in WKY rats. The urinary albumin-to-creatinine ratio (ACR) was significantly greater in the Sed-SHR compared with Sed-WKY group, and the ACR was significantly reduced in the Ex-SHR compared with Sed-SHR group. There was no significant difference in the urinary ACR between exercised and sedentary WKY rats.

Effects of exercise on NO_x and nitric oxide synthase activity

Plasma and urinary NO_x were significantly greater in the Sed-SHR compared with Sed-WKY group, with exercise significantly increasing plasma and urinary NO_x in both strains (Fig. 2). Nitric oxide synthase activity in the renal cortex, outer medulla, inner medulla and aorta was significantly greater in the Sed-SHR compared with Sed-WKY group (by 51%, 45%, 37% and 39%, respectively; Fig. 3). Exercise significantly increased NOS activity in the renal cortex, outer medulla, inner medulla and aorta by 65%, 73%, 81% and 110%, respectively, in WKY rats and by 49%, 50%, 28% and 41%, respectively, in SHR (Fig. 3).

Effects of exercise on nitric oxide synthase expression and eNOS phosphorylation

Immunoblots comparing the expression of eNOS protein in kidney sections and the aorta are shown in Fig. 4. Levels of eNOS

Table 1 Effects of exercise training on biochemical parameters in Wistar–Kyoto and spontaneously hypertensive rats

	Wistar–Kyoto rats		Spontaneously hypertensive rats	
	Sedentary	Exercised	Sedentary	Exercised
Plasma				
TC (mg/dL)	89.0 ± 4.6	79.9 ± 6.6	64.4 ± 2.1	61.8 ± 2.4
TG (mg/dL)	32.2 ± 3.3	41.8 ± 6.0	53.0 ± 4.4	61.4 ± 4.7
FFA (mEq/L)	0.243 ± 0.024	0.193 ± 0.018	0.187 ± 0.013	0.203 ± 0.016
Glucose (mg/dL)	177 ± 5	164 ± 6	200 ± 6	188 ± 6
BUN (mg/dL)	18.9 ± 0.6	14.4 ± 0.6	17.9 ± 0.6	17.3 ± 0.8
Cr (mg/dL)	0.188 ± 0.014	0.172 ± 0.017	0.167 ± 0.012	0.113 ± 0.021*
Noradrenaline (ng/mL)	1.88 ± 0.31	1.64 ± 0.18	1.33 ± 0.08	1.58 ± 0.28
Renin activity (ng/mL per h)	4.34 ± 0.70	3.06 ± 0.43	6.14 ± 0.65	5.50 ± 1.34
Urine				
Sodium (g/L)	3.88 ± 0.16	3.46 ± 0.18	4.06 ± 0.17	3.88 ± 0.29
ACR (mg/g Cr)	65.3 ± 11.2	43.7 ± 5.0	169 ± 29††	44.6 ± 2.9**
Noradrenaline (mg/day)	3.26 ± 0.43	2.02 ± 0.57	2.92 ± 0.63	1.38 ± 0.11
Cr clearance (mL/min)	2.15 ± 0.31	2.45 ± 0.42	2.50 ± 0.51	4.57 ± 0.69*

Data are mean ± SEM (n = 6 rats per group). ††$P < 0.01$ compared with the sedentary Wistar–Kyoto rats; *$P < 0.05$, **$P < 0.01$ compared with the sedentary group in the same rat strain.

TC, total cholesterol; TG, triglycerides; FFA, free fatty acids; BUN, blood urea nitrogen; ACR, albumin : creatinine ratio; Cr, creatinine.

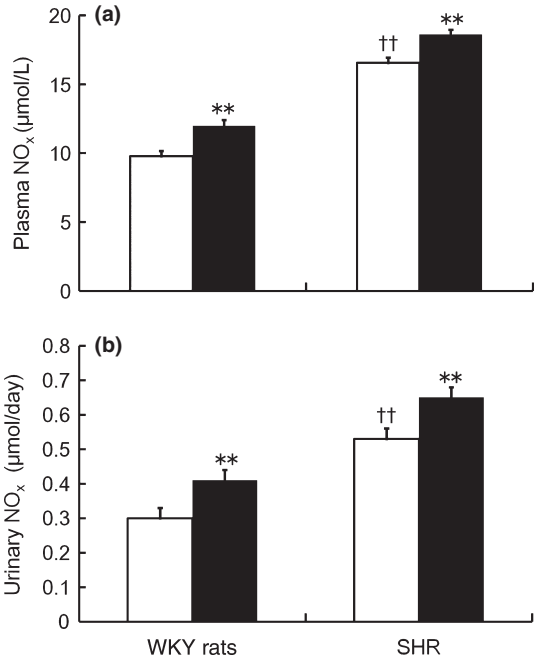

Fig. 2 Effects of exercise training on the (a) plasma and (b) urinary nitrate/nitrite (NO_x) in Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Wistar–Kyoto rats and SHR were randomized into sedentary (□) and exercised (■) groups. Data are the mean ± SEM (n = 6 rats per group). ††$P < 0.01$ compared with sedentary WKY rats; **$P < 0.01$ compared with the sedentary group in the same strain.

protein in the renal cortex, outer medulla, inner medulla and the aorta were significantly greater in the Sed-SHR than Sed-WKY group (by 126%, 96%, 48% and 65%, respectively). Exercise significantly increased levels of eNOS protein in the renal cortex, outer medulla, inner medulla and aorta by 92%, 98%, 71% and 90%, respectively, in WKY rats and by 99%, 49%, 32% and 62%, respectively, in SHR compared with their respective sedentary groups. Exercise also significantly decreased the ratio of p-eNOS(Ser^{1177}) : total eNOS in the renal cortex, outer medulla and inner medulla by 43%, 70% and 69%, respectively, in WKY rats and by 54%, 30% and 29%, respectively, in SHR (Fig. 5). Furthermore, exercise significantly decreased the ratio of p-eNOS (Thr^{495}) : total eNOS in the renal cortex, outer medulla and inner medulla by 69%, 79% and 94%, respectively, in WKY rats and by 90%, 57% and 35%, respectively, in SHR (Fig. 6). The decrease in eNOS phosphorylation at Thr^{495} was significantly greater than that at Ser^{1177} in the renal cortex, outer medulla and inner medulla of both SHR and WKY rats ($P < 0.01$ for all).

Immunoblots comparing the expression of nNOS protein in kidney sections and the aorta are shown in Fig. 7. Levels of nNOS-α protein in the renal cortex, outer medulla, inner medulla and aorta were significantly greater in the Sed-SHR than Sed-WKY group by 63%, 39%, 70% and 51%, respectively. Exercise significantly increased nNOS-α protein levels in the renal cortex, outer medulla, inner medulla and aorta by 35%, 34%, 65% and 62%, respectively, in WKY rats and by 18%, 13%, 31% and 31%, respectively, in SHR, compared with their respective sedentary groups. In contrast, there were no significant differences in levels of renal nNOS-β protein between exercised and sedentary rats of either strain, except for the inner medulla of SHR, where exercise significantly decreased levels of nNOS-β protein by 64%. There were no significant differences in levels of renal iNOS protein between exercised and sedentary rats of either strain (data not shown).

Effects of exercise on thiobarbituric acid-reactive substances levels and NADPH oxidase activity

There were no significant differences in plasma TBARS levels between exercised and sedentary rats of either strain (Fig. 8a). Urinary TBARS levels were significantly greater in the Sed-SHR than Sed-WKY group and exercise significantly decreased urinary TBARS level in SHR (Fig. 8b). Exercise resulted in a significant decrease in renal NADPH oxidase activity in the renal cortex and outer medulla of SHR, but significantly increased renal NADPH oxidase activity in WKY rats (Fig. 8c,d).

DISCUSSION

Despite the beneficial effects of exercise on the cardiovascular system, the renal effects of exercise have not been fully clarified. In the present study, we found that exercise significantly attenuated the development of hypertension, decreased urinary albumin excretion, TBARS levels and renal NADPH oxidase activity, and increased creatinine clearance in SHR. Furthermore, exercise significantly increased NOS activity and eNOS and nNOS expression in the kidneys and aorta of both SHR and WKY rats. These data suggest that the upregulation of renal NOS by exercise may contribute, at least in part, to the antihypertensive and renal protective effects in SHR. In contrast with previous

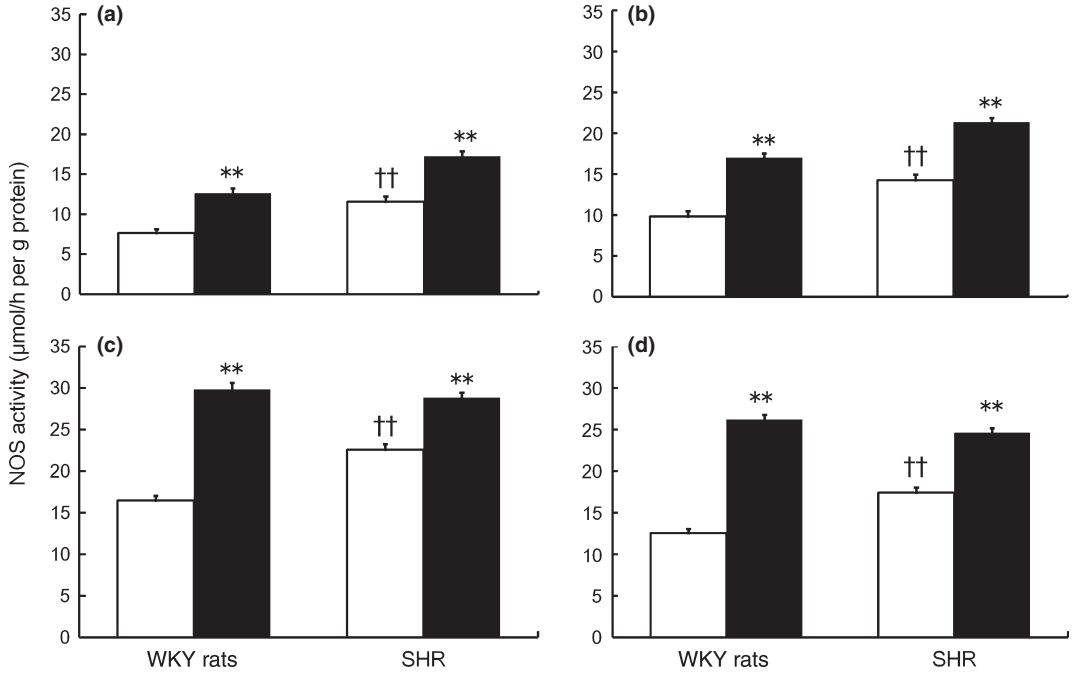

Fig. 3 Effects of exercise training on nitric oxide synthase (NOS) activity in the (a) renal cortex, (b) outer medulla, (c) inner medulla and (d) aorta of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Wistar–Kyoto rats and SHR were randomized into sedentary (□) and exercised (■) groups. Data are the mean ± SEM (n = 6 rats per group). $^{\dagger\dagger}P < 0.01$ compared with sedentary WKY rats; $^{**}P < 0.01$ compared with the sedentary group in the same strain.

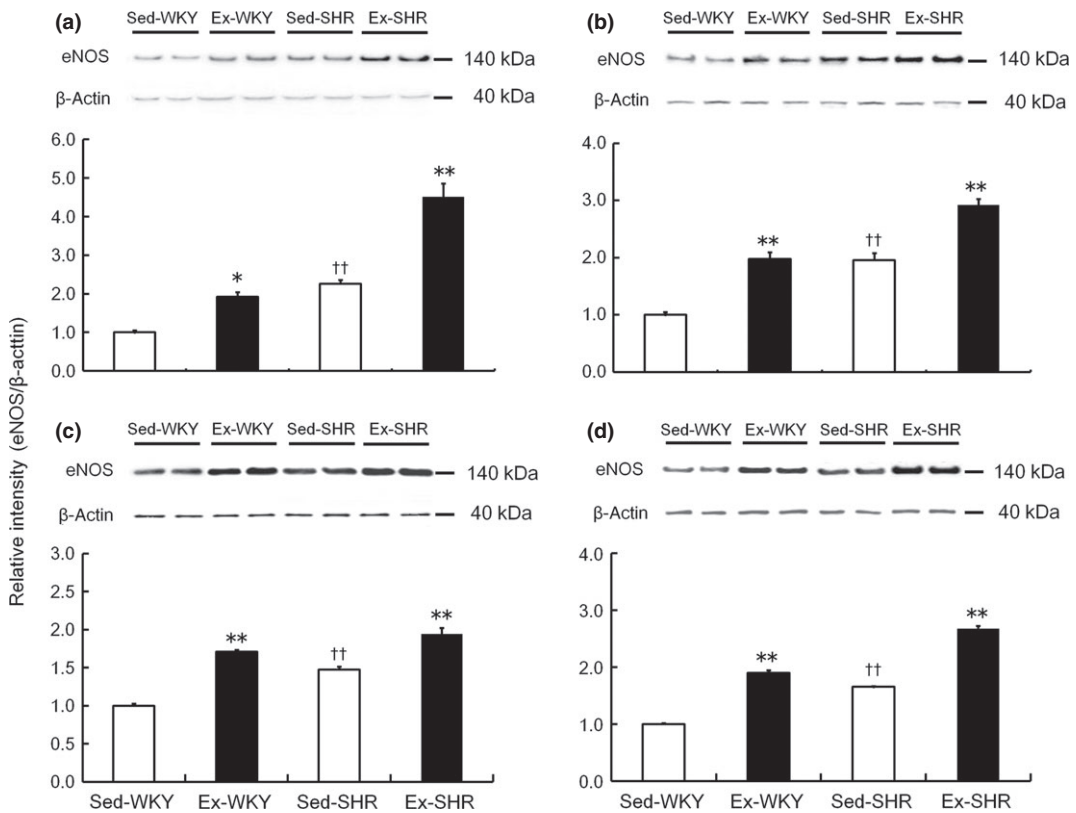

Fig. 4 Effects of exercise training on endothelial nitric oxide synthase (eNOS) protein expression in the (a) renal cortex, (b) outer medulla, (c) inner medulla and (d) aorta of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Wistar–Kyoto rats and SHR were randomized into sedentary (Sed-WKY, Sed-SHR; □) and exercised (Ex-WKY, Ex-SHR; ■) groups. The top panels show representative immunoblots from the different groups. The intensities of the eNOS bands (140 kDa) for each protein were normalized against that of β-actin (40 kDa) and the intensity of the band in the Sed-WKY group was assigned a value of 1. Data are the mean ± SEM (n = 6 rats per group). $^{\dagger\dagger}P < 0.01$ compared with Sed-WKY rats; $^{**}P < 0.01$ compared with the sedentary group in the same strain.

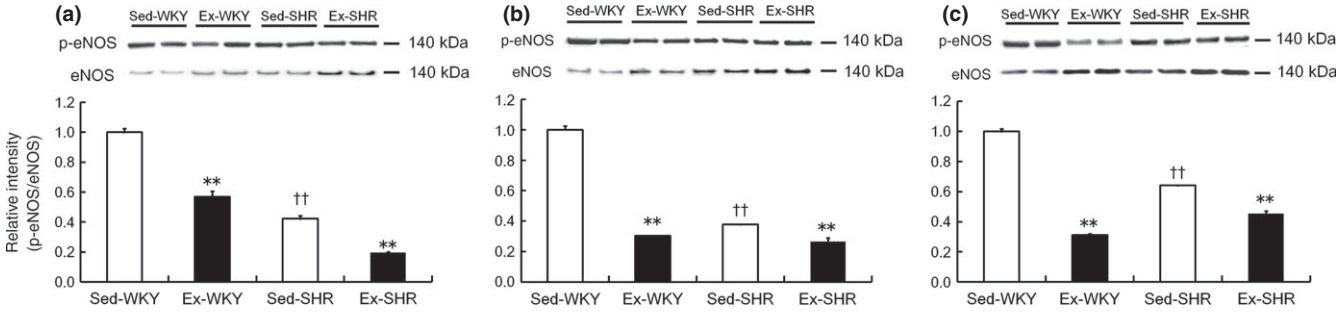

Fig. 5 Effects of exercise training on the phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser^{1177} in the (a) renal cortex, (b) outer medulla and (c) inner medulla of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Wistar–Kyoto rats and SHR were randomized into sedentary (Sed-WKY, Sed-SHR; □) and exercised (Ex-WKY, Ex-SHR; ■) groups. Levels of phosphorylated (p-) eNOS(Ser^{1177}) were determined by immunoblot analysis. The top panels show representative immunoblots from the different groups. The ratio of the relative intensities of the bands for p-eNOS to total eNOS was calculated, with the ratio in the Sed-WKY group assigned a value of 1. Data are the mean ± SEM (n = 6 rats per group). $^{††}P < 0.01$ compared with sedentary WKY rats; $^{**}P < 0.01$ compared with the sedentary group in the same strain.

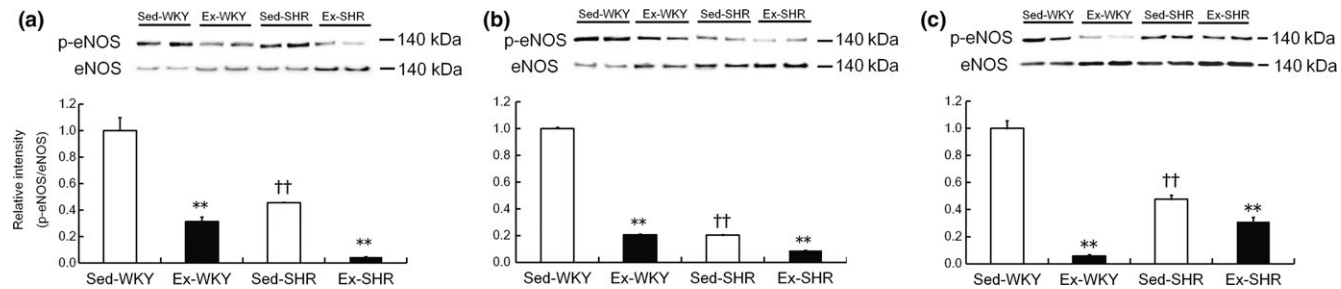

Fig. 6 Effects of exercise training on the phosphorylation of endothelial nitric oxide synthase (eNOS) at Thr^{495} in the (a) renal cortex, (b) outer medulla and (c) inner medulla of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Wistar–Kyoto rats and SHR were randomized into sedentary (Sed-WKY, Sed-SHR; □) and exercised (Ex-WKY, Ex-SHR; ■) groups. Levels of phosphorylated (p-) eNOS(Thr^{495}) and total eNOS were determined by immunoblot analysis. The top panels show representative immunoblots from the different groups. The ratio of the relative intensities of the bands for p-eNOS to total eNOS was calculated, with the ratio in the Sed-WKY group assigned a value of 1. Data are the mean ± SEM (n = 6 rats per group). $^{††}P < 0.01$ compared with sedentary WKY rats; $^{**}P < 0.01$ compared with the sedentary group in the same strain.

studies,[37,38] in the present study the SBP in SHR and WKY rats was high and there was a moderate antihypertensive effect of exercise. It is possible that the young age of the rats (5–13 weeks), the duration and speed of the treadmill exercise, or the heating of rats by equipment required to conduct tail-cuff measurements of SBP may have had an effect on the high SBP.

Both Ser^{1177} and Thr^{495} are key residues involved in the phosphorylation and dephosphorylation networks that influence eNOS activity.[39] Phosphorylation at Ser^{1177} stimulates eNOS activity, whereas phosphorylation at Thr^{495} inhibits eNOS activity.[39] Our results demonstrating that inhibition of eNOS phosphorylation at Thr^{495} was significantly greater than that at Ser^{1177} may be related to upregulated NOS activity in the kidney of SHR and WKY rats. Further studies are required to clarify the mechanisms by which exercise inhibits eNOS phosphorylation in the kidney.

The present study showed that exercise significantly induced nNOS-α and eNOS expression in the kidney of both SHR and WKY rats. Exercise has been reported to restore the reduced expression of nNOS and to suppress the overexpression of angiotensin II and AT_1 receptors in the carotid body of rabbits with chronic heart failure (CHF).[40] Furthermore, in rats with CHF, exercise restored decreased nNOS expression in the paraventricular nucleus and contributed to improvements in exaggerated renal sympathetic nerve activity.[41] In the present study, plasma renin activity and urinary noradrenaline in exercised SHR were reduced, albeit not significantly, compared with values in sedentary SHR. The lack of any significant effects of exercise on plasma renin activity and noradrenaline levels may be due to the fact that levels of the renin–angiotensin–aldosterone system (RAAS) and sympathetic nerve activity in the SHR in the present study were not as high as in CHF. In rat models, it has been established that inhibitors of NOS can elevate blood pressure,[11] whereas administration of L-arginine lowers blood pressure.[12] Therefore, renal NO may contribute, at least in part, to the antihypertensive effects of chronic exercise. Further studies are necessary to identify the antihypertensive mechanisms of exercise-induced NOS expression in the kidney.

Although the differing roles of the various nNOS isoforms remain contentious, renal expression of nNOS-α protein has been reported to decrease in rats with CRF,[36] whereas renal expression of nNOS-β protein increases in CRF rats,[36] ageing rats[42] and rats fed a high-salt diet.[43] In the present study, exercise significantly increased the expression of nNOS-α, but not nNOS-β, in kidney sections of SHR and WKY rats, except in the inner medulla of SHR, suggesting renal protective effects of exercise.

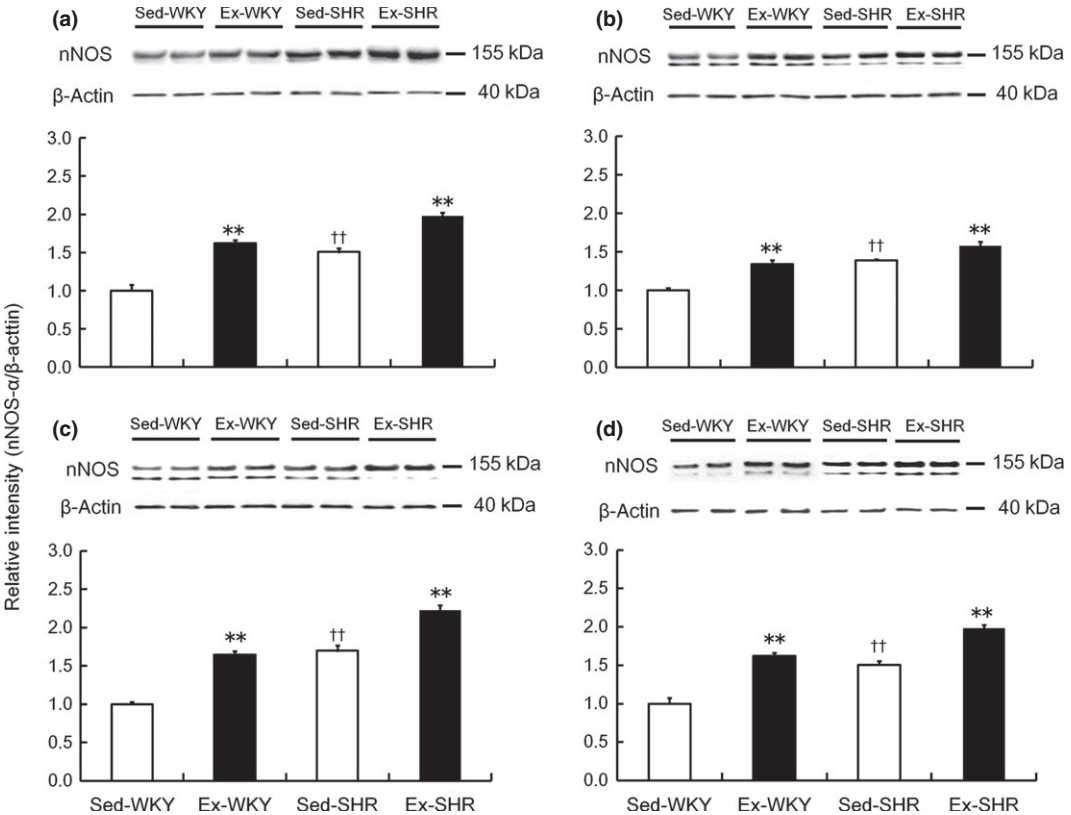

Fig. 7 Effects of exercise training on neuronal nitric oxide synthase (nNOS)-α protein expression in the (a) renal cortex, (b) outer medulla, (c) inner medulla and (d) aorta of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Wistar–Kyoto rats and SHR were randomized into sedentary (Sed-WKY, Sed-SHR; □) and exercised (Ex-WKY, Ex-SHR; ■) groups. The top panels show representative immunoblots from the different groups. The intensities of the nNOS-α bands (155 kDa) for each protein were normalized against those for β-actin (40 kDa), with the intensity of the band in the Sed-WKY group assigned a value of 1. Data are the mean ± SEM (n = 6 rats per group). $^{††}P < 0.01$ compared with sedentary WKY rats; $^{**}P < 0.01$ compared with the sedentary group in the same strain.

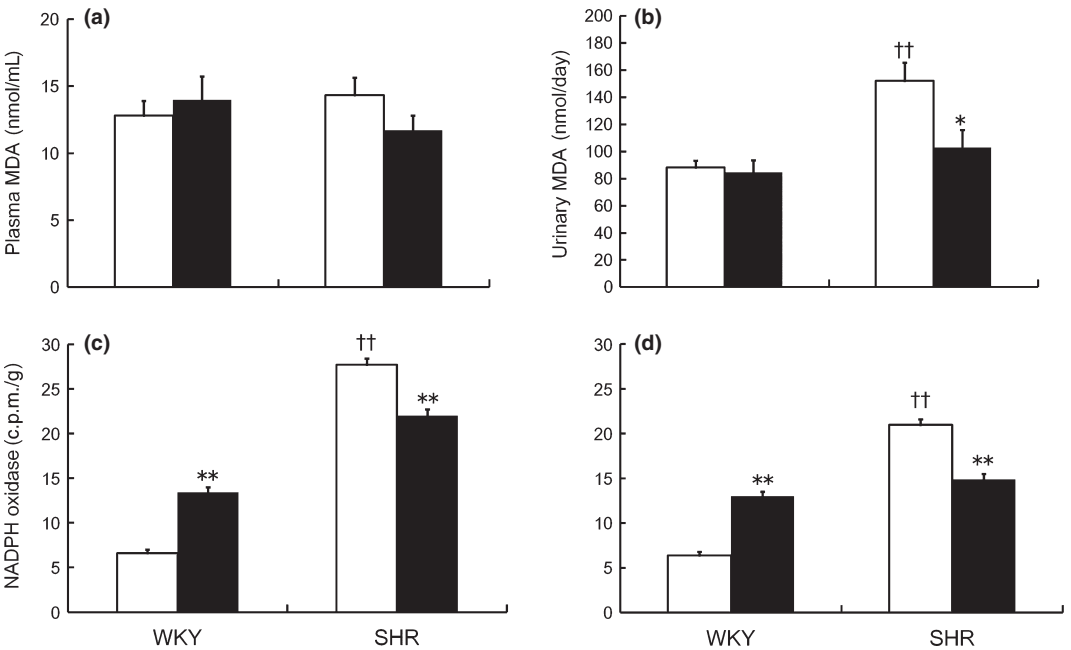

Fig. 8 Effects of exercise training on (a) plasma and (b) urinary thiobarbituric acid-reactive substances (TBARS) levels, expressed as malondialdehyde (MDA) concentrations, and renal NADPH oxidase activity in the (c) renal cortex and (d) outer medulla in Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Wistar–Kyoto rats and SHR were randomized into sedentary (□) and exercised (■) groups. Data are the mean ± SEM (n = 6 rats per group). $^{††}P < 0.01$ compared with sedentary WKY rats; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the sedentary group in the same strain.

We found that concentrations of plasma and urinary NO_x in Sed-SHR were significantly greater than in Sed-WKY rats. Nitric oxide production was reported previously to increase in young SHR, indicative of progressive hypertension, but decrease in old SHR.[44] The increased NO production in SHR may have a negative feedback on elevated oxidative stress and sympathetic overactivity, which likely results in alleviation of excessive blood pressure elevation.[45] Therefore, exercise may further enhance the protective effects of NOS in SHR.

Increased production of superoxide in the medullary thick ascending limb (TAL) was reported to attenuate NO, leading to reduced medullary blood flow in Dahl salt-sensitive rats.[26] Thus, elevated eNOS and nNOS in the outer medulla in response to exercise are assumed to contribute to improvements in medullary blood flow, partly leading to enhancement of the glomerular filtration rate (GFR). In addition, the induction of nNOS by exercise in the renal cortex supports the hypothesis that increased nNOS in the macula densa is involved, in part, in regulation of TGF and the RAAS.[17,24] Previously, we reported that exercise had renal protective effects in rats with CRF[5,6] or diabetic nephropathy,[7] as well as in fructose-fed SHR.[8] Consistent with the present study, Agarwal *et al.*[46] reported that exercise increased GFR and improved glomerular injury with increased eNOS expression in the renal cortex of SHR. Furthermore, in that study exercise prevented renal oxidative stress and inflammation and modulated components of the RAAS in SHR.[46]

In the present study, we also found that exercise upregulated eNOS and nNOS expression in the renal medulla, suggesting that exercise-induced NO in the renal medulla may contribute to the inhibition of NaCl reabsorption in the TAL and osmotic water permeability in the collecting duct,[16,17] as well as to improvements in medullary blood flow.[26] Furthermore, exercise increased nNOS-α, but not nNOS-β, expression in the kidney, suggesting renal protective effects of exercise[36,42,43] and that the increased nNOS induced by exercise in the macula densa may contribute to the regulation of TGF and the RAAS.[17,24] We also found that exercise increased renal NOS activity and plasma and urinary NO_x levels and decreased eNOS phosphorylation at Ser^{1177} and Thr^{495} in the kidney. Furthermore, we found no significant difference in plasma TBARS levels between exercised and sedentary rats in either strain, but significantly decreased urinary TBARS levels in exercised SHR, suggesting that exercise may have an effect on lipid peroxidation in the kidney of SHR. A key finding of the present study is that exercise significantly reduces renal NADPH oxidase activity in SHR but increases it in WKY rats. These findings suggest that exercise suppresses oxidative stress under conditions of high oxidative stress in SHR, but upregulates oxidative stress under conditions of normal oxidative stress in WKY rats. Furthermore, these results raise the possibility that exercise-induced NO may suppress the elevated oxidative stress in SHR, whereas exercise-induced NO may increase oxidative stress in WKY rats. One of the keys to explaining this discrepancy may be the fact that NO bioavailability is greater in SHR than in WKY rats. The induction of low levels of NO by exercise under normal conditions in WKY rats may upregulate renal oxidative stress, but these changes have no effect on blood pressure and renal function, whereas the induction of high levels of NO by exercise under hypertensive conditions in SHR may contribute, at least in part, to the suppression of renal high oxidative stress and renal protective effects. However, the changes in renal NO and oxidative stress are not sufficient to explain the antihypertensive and renal protective effects of exercise. Further studies are needed to identify alternative mechanisms.

In conclusion, we found that exercise significantly lowers SBP, decreases urinary albumin excretion, TBARS levels and renal NADPH oxidase activity, and upregulates renal eNOS and nNOS expression and NOS activity in SHR. Overall, these data suggest that exercise may be a novel therapeutic approach for preventing the development of renal dysfunction in hypertensive patients via the upregulation of renal NOS.

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