

# Effects of chronic *N*-acetylcysteine treatment on the actions of peroxynitrite on aortic vascular reactivity in hypertensive rats

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**Background** Peroxynitrite (ONOO<sup>-</sup>), the product of superoxide and nitric oxide, seems to be involved in vascular alterations in hypertension.

**Objectives** To evaluate the effects of ONOO<sup>-</sup> on endothelium-dependent and independent aortic vascular responsiveness, oxidized/reduced glutathione balance (GSSG/GSH), malondialdehyde aortic content, and the formation of 3-nitrotyrosine (3-NT), a stable marker of ONOO<sup>-</sup>, in *N*-acetylcysteine (NAC)-treated normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR).

**Results** In SHR only, NAC significantly reduced heart rate and systolic, but not diastolic, blood pressure. It also improved endothelium-dependent aortic relaxation in SHR, but not after exposure to ONOO<sup>-</sup>. Endothelium-dependent and independent aortic relaxations were markedly impaired by ONOO<sup>-</sup> in both strains of rat. NAC partially protected SHR against the ONOO<sup>-</sup>-induced reduction in endothelium-independent relaxation. Aortic GSSG/GSH ratio and malondialdehyde, which were higher in SHR than in WKY rats, showed a greater increase in SHR after exposure to ONOO<sup>-</sup>. NAC decreased GSSG/GSH and malondialdehyde in both strains of rat before and after exposure to ONOO<sup>-</sup>. The 3-NT concentration, which was similar in both strains of rat under basal conditions, was greater in SHR than in WKY rats after the addition of ONOO<sup>-</sup>, with a reduction only in NAC-treated SHR.

**Conclusions** These findings suggest an increased

vulnerability of SHR aortas to the effects of ONOO<sup>-</sup> as compared with those of WKY rats. The selective improvements produced by NAC, in systolic arterial pressure, heart rate, aortic endothelial function, ONOO<sup>-</sup>-induced impairment of endothelium-independent relaxation, aortic GSSG/GSH balance, malondialdehyde content and 3-NT formation in SHR suggest that chronic administration of NAC may have a protective effect against aortic vascular dysfunction in the SHR model of hypertension. *J Hypertens* 19:1233–1244   2001 Lippincott Williams & Wilkins.

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## Introduction

Free radicals can participate in physiological mechanisms, but they also exert deleterious effects when produced in excess, leading to the development of certain cardiovascular diseases [1]. An imbalance between the generation of free radicals and the antioxidant buffer capacity have been suggested to account for the accelerated inactivation of the endothelium-derived relaxing factor, nitric oxide, thereby presum-

ably contributing to the greater peripheral vascular resistance seen in human [2] and experimental hypertension [3]. Oxidative imbalance may be responsible for the generation, in vascular tissue, of peroxynitrite anion (ONOO<sup>-</sup>) from the interaction between nitric oxide and superoxide anion [4]. ONOO<sup>-</sup> generation could be facilitated in hypertension; however, little is known about the vascular and cellular effects of ONOO<sup>-</sup> in hypertension. In the normotensive situation, the vascular effects of ONOO<sup>-</sup> have been found to be varied and inconclusive, and differences in vascular and cellular response have been reported. In

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fact, along with the cytotoxic actions of  $\text{ONOO}^-$ , such as lipid peroxidation [5] and the alteration of protein structure and function by the nitration of tyrosine residues [6] or oxidation of thiol residues [7,8], recent reports indicate that  $\text{ONOO}^-$  exerts cytoprotective effects [9,10].

We therefore decided to investigate the aortic vascular effects of  $\text{ONOO}^-$  on endothelium-dependent and -independent vasodilators in the spontaneously hypertensive rat (SHR), a genetic model of hypertension that mimics human essential hypertension, compared with those in its normotensive control, the Wistar-Kyoto (WKY) rat. Cellular effects of  $\text{ONOO}^-$  were also investigated by measuring the aortic content of reduced (GSH) and oxidized (GSSG) glutathione and of malondialdehyde, an index of tissue lipid peroxidation; in an organ bath experiment, we also measured the formation of free 3-nitrotyrosine (3-NT), a stable marker of  $\text{ONOO}^-$  generation. In parallel studies, a subgroup of rats from each strain was treated by chronic oral administration of *N*-acetylcysteine (NAC), a known antioxidant [11], to evaluate the possible existence of different protective effects against  $\text{ONOO}^-$  under normotensive and hypertensive conditions.

## Methods

### Drugs

All drugs and chemical components of solutions were purchased from Sigma Chemical Company (St Louis, Missouri, USA) except for 2-vinylpyridine, which was purchased from Sigma-Aldrich s.r.l. (Milan, Italy) and peroxynitrite ( $\text{ONOO}^-$ ), which was synthesized as described below.

### Animals and treatment

Rats were treated in accordance with the Canadian Council on Animal Care guidelines and the procedures were approved by the Local Institutional Animal Ethics Committee. Seventy-two 13-week-old male SHR and WKY rats (Charles River Laboratories, St Constant, Québec, Canada, and Calco, Italy) were housed two per cage under a 12 h light–12 h darkness cycle, at constant humidity and temperature, with free access to standard laboratory rat chow (Basal Purified Diet 5755C, Purina Mills Inc. St Louis, Missouri, USA) and drinking water. After a few days of acclimatization, rats were randomly divided into control groups (SHR,  $n = 18$ ; WKY rats,  $n = 18$ ) and NAC-treated (SHR-NAC,  $n = 18$ , WKY-NAC,  $n = 18$ ) groups, receiving NAC in their drinking water for a period of 2 weeks. NAC was dissolved in water to give a calculated daily intake of 4 g/kg body weight. Because of its poor oral bioavailability (4–10%) [11], a high dose of NAC was used. No differences in daily water intake and body weight were observed among groups.

Effects of NAC on systolic and diastolic arterial pressures and heart rate were measured in all groups ( $n = 12$  rats for each group) by a computerized analysis program (Acknowledge 3.0, Harvard Apparatus) in chronically cannulated conscious and unrestrained rats. The femoral artery was cannulated with polyethylene tubing (PE-10) welded to a PE-50 catheter after the rats were anaesthetized with sodium pentobarbitone (50 mg/kg body weight intraperitoneally). The catheter, filled with heparinized saline, was positioned in the abdominal aorta, tunnelled subcutaneously, extruded at the back of the neck, and protected from the rat by insertion into a stainless steel tether. After the procedure, the rats were placed in individual cages. The arterial catheter was coupled to a pressure transducer (Statham P231D, Gould Statham Inc.), and the signal was amplified and recorded by a Biopac data-acquisition system (MP100WS, Harvard Apparatus Canada).

### Peroxynitrite synthesis

$\text{ONOO}^-$  and decomposed  $\text{ONOO}^-$  were synthesized by the method described by Beckman *et al.* [6]. Briefly, ice-cold solutions of 0.6 mol/l  $\text{NaNO}_2$  and 0.6 mol/l HCl plus 0.7 mol/l  $\text{H}_2\text{O}_2$  were pumped at a flow rate of 20 ml/min into a Y junction to form peroxynitrous acid; this reaction was quenched by pumping 1.5 mol/l NaOH at the same rate into a sidearm connection at the end of the tubing. Residual  $\text{H}_2\text{O}_2$  was removed by passage over a  $1 \times 8$  cm column filled with 4 g granular  $\text{MnO}_2$ . The solution was then frozen at  $-20^\circ\text{C}$  for as long as 1 week.  $\text{ONOO}^-$  formed an intense yellow top layer as a result of frost fractionation; this layer contained 120–170 mmol/l  $\text{ONOO}^-$  as determined by absorbance at 302 nm (molar absorptivity  $\epsilon = 1670$  (mol/l) $^{-1}$  cm $^{-1}$ ). Decomposed  $\text{ONOO}^-$  was prepared in a similar fashion, except that the solutions of  $\text{NaNO}_2$  and  $\text{H}_2\text{O}_2$  in HCl were not quenched in NaOH – which was, however, added 5 min later when the  $\text{ONOO}^-$  was completely decomposed. The concentration of  $\text{ONOO}^-$  in the latter solution was verified by ultraviolet spectroscopy and was almost undetectable. Just before the experiments, the stock solution of  $\text{ONOO}^-$  in 1.5 mol/l NaOH (which had been aliquoted, stored at  $-80^\circ\text{C}$  and protected from light) was diluted 1 : 1000 in ice-cold purified Milli-Q water (Millipore Water Purification System, Etobicoke, Ontario, Canada) to avoid the trace presence of metal contaminants, giving a final working solution of 4 mmol/l of  $\text{ONOO}^-$  in 1.5 mmol/l NaOH, verified by ultraviolet spectroscopy. Both  $\text{ONOO}^-$  and decomposed  $\text{ONOO}^-$  were tested in all the experiments because, even if  $\text{MnO}_2$  had been used to remove the residual  $\text{H}_2\text{O}_2$ , the working solution may have contained traces of  $\text{H}_2\text{O}_2$  that could activate vascular or cellular effects. In addition, the use of  $\text{MnO}_2$  could be a potential source, even if rarely so, of metal contamination. The vehicle may have had traces of  $\text{H}_2\text{O}_2$  and be contaminated with

manganese, therefore decomposed ONOO<sup>-</sup> was tested to exclude possible interference with the effects of ONOO<sup>-</sup>.

### 3-Nitrotyrosine analysis

Because ONOO<sup>-</sup> at physiological pH is highly unstable, before the beginning of each experiment we always verified the reproducibility of the efficacy of ONOO<sup>-</sup> in nitrosating L-tyrosine ( $3 \times 10^{-7}$  mol/l) added to the Krebs–Henseleit buffer in the organ bath without aortic rings, by measuring free 3-NT generation. In previous experiments, no detectable amount of 3-NT was found in samples obtained from an organ bath filled with the L-tyrosine-free buffer. Free 3-NT in the organ bath was also measured in the presence of aortic rings, thus allowing for the release of ONOO<sup>-</sup> by aortic tissue and the antioxidant capacity of aortic rings to scavenge the exogenously added ONOO<sup>-</sup>.

Samples (4 ml) of L-tyrosine buffer were therefore collected from the organ bath chamber before and 1 min after the addition of ONOO<sup>-</sup>. After the extraction, 3-NT was concentrated 40-fold to be in the detection range of this substance, which is above 0.15 µmol/l using high-performance liquid chromatography (HPLC) coupled to an ultraviolet detection system as we described previously [12]. Briefly, columns for solid-phase extraction were used; after the pH of the solution had been adjusted to 5.65 (isoelectric point of the L-tyrosine), samples were injected through a conditioned C 18 cartridge (Bond Elut LRC 1211-3027, Varian, Palo Alto, California, USA) and 3-NT was finally eluted with 1 ml of HPLC-grade methanol. After evaporation of the solvent in a speed vacuum, the extract was redissolved in a final volume of 100 µl (equal volumes of methanol and purified water). Thirty-microlitre aliquots were injected into the HPLC system by a SIL-9A autoinjector (Shimadzu Scientific Instruments Inc., Columbia, Maryland, USA). The formation of 3-NT from L-tyrosine was analysed by separation in HPLC (Primesphere C18 5 µm, 25 cm × 4.6 mm, Phenomenex Inc., Torrance, California, USA), detected by ultraviolet absorbance at 274 nm (Shimadzu SPD-6AV) and recorded on a chromatography Data System (Shimadzu C-R6A). The mobile phase, consisting of 220 mmol/l KH<sub>2</sub>PO<sub>4</sub>–H<sub>3</sub>PO<sub>4</sub> (pH 3.01), 0.86 mmol/l sodium dodecyl sulphate and 10% methanol (v/v), was pumped by an isocratic system at a flow rate of 0.8 ml/min. Peaks of *p*-tyrosine, *m*-tyrosine, *o*-tyrosine and 3-NT were identified on the spectrum of the coelution and quantified by peak height using external standards.

### GSH and GSSG measurements

The aortic content of GSH and of GSSG was measured in aortas by the enzymatic recycling technique of Griffith *et al.* [13]. In short, sections of aortas, before

and after 1 min of exposure to ONOO<sup>-</sup>, were washed in chilled buffer, rapidly frozen and stored at –80°C until required for analysis, which was performed within 24 h. Later, tissues were rapidly homogenized in a volume of 500 µl of a chilled solution of 5% 5-sulphosalicylic acid and ethylenediamine tetra-acetic acid (EDTA, 0.5 mmol/l), sonicated (15 s four times), centrifuged (10 000 *g* for 5 min at 4°C), and the supernatant collected. Seven hundred microlitres of a buffer containing sodium phosphate (143 mmol/l), Na<sub>4</sub>-EDTA (6.3 mmol/l) and NADPH (0.248 mg/ml), 100 µl of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 6 mmol/l) solution, 175 µl of distilled water and 25 µl of sample supernatant were pipetted into each cuvette. The cuvette was warmed at 30°C in a water bath for 15 min. Ten microlitres of GSSG reductase (266 U/ml) were then mixed in, to initiate the assay. The rate of formation of 5-thio-2-nitrobenzoic acid induced by the reaction of GSH in the supernatant and DTNB was followed by ultraviolet absorbance at 412 nm (Beckman Coulter, Inc. DU 640B Bio-Spectrophotometer, Fullerton, California, USA). The amount of GSH was determined from a standard curve in which the concentration of GSH was plotted against the rate of change of absorbance at 412 nm. The assay was also monitored by evaluation of the NADPH disappearance curve at a wavelength of 340 nm. GSSG was determined after a 60 min derivatization of supernatant samples (100 µl) with 2-vinylpyridine (2 µl), which links to GSSG, followed by the same procedure described above. Values of GSH and GSSG were expressed on the basis of the protein content in the supernatant as determined by the Bradford method [14].

### Aortic lipid peroxidation: assay of malondialdehyde

Malondialdehyde content, an index of lipid peroxidation, was measured in aortas by the modification of the method described by Yagi *et al.* [12,15], involving the fluorimetric detection of malondialdehyde. In short, sections of thoracic aortas, before and after 1 min of exposure to ONOO<sup>-</sup>, were homogenized in chilled phosphate buffer (KH<sub>2</sub>PO<sub>4</sub> 50 mmol/l, pH 7.40), using a homogenizer (Ultraturrax T8, Janke & Kunke, Staufen, Germany), three times for 15 s, with a 30 s cooling interval. After a filtration, the homogenate was centrifuged (1000 *g* for 10 min at 4°C) and the supernatant was rapidly frozen and stored at –80°C until required for analysis, which was performed within 1 week. To avoid amplification of peroxidation during storage (–80°C) and the assay, 5 µl of 3 mmol/l butylated hydroxytoluene and 5 µl of 2 mmol/l desferoxamine were added to the phosphate extraction buffer (500 µl). Two millilitres of 4 mol/l H<sub>2</sub>SO<sub>4</sub> and 0.25 ml of 10% (wt/vol) phosphotungstic acid were added to a tube containing 100 µl of tissue supernatant; this mixture was left at room temperature for 10 min before being

centrifuged at 2000 *g* for 10 min. The supernatant was then discarded and the pellet resuspended in a solution of 1 ml of 4 mol/l H<sub>2</sub>SO<sub>4</sub> and 0.15 ml of 10% (wt/vol) phosphotungstic acid, followed by a second centrifugation (at 2000 *g* for 10 min). The pellet was then resuspended in 2 ml of water to which 0.5 ml of a mixture of equal volumes of 0.67% thiobarbituric acid aqueous solution and glacial acetic acid was added. This solution was heated at 95°C for 60 min in a water bath. After the tubes had been cooled on ice, 2.5 ml of *n*-butanol was added and the mixture was agitated on a vortex for 30 s. Finally, the solution was centrifuged again (2000 *g* for 10 min at room temperature) and the butanol layer transferred for fluorimetric detection (Fluorimeter Model 650, Perkins-Elmer, Norwalk, Connecticut, USA) at a wavelength of 553 nm with excitation at 515 nm against an appropriate blank. A standard curve of malondialdehyde was prepared from tetraethoxypropane, which yielded equimolar amounts of malondialdehyde. Values of malondialdehyde were expressed as nmol/mg protein content in the supernatant [14].

#### Aortic preparations

Experiments were performed on aortic rings prepared from the descending thoracic aorta of the rats at 15 weeks of age, as we described previously [16]. Immediately after the animals were killed, the thoracic aorta was removed and cleaned of all fat and connective tissue and cut in 4 mm rings. Endothelium-intact aortic rings were mounted into 20 ml volume organ baths filled with a Krebs-Henseleit buffer of the following millimolar composition: NaCl 118, KCl 4, CaCl<sub>2</sub> 2.5, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgSO<sub>4</sub> 1, NaHCO<sub>3</sub> 24, D-glucose 11. The preparations were then connected to a strain gauge (Grass FT03) and isometric tension was recorded on a Grass polygraph. The rings were stretched to an optimal 2 g resting tension and after allowed to equilibrate for 30 min. before experimentation. All the aortic rings were then pre-contracted with phenylephrine at a concentration of  $3 \times 10^{-7}$  mol/l, giving similar contractile responses (70% of the respective maximal response) in endothelium-intact WKY rats and SHR, whereas a greater sensitivity was observed in endothelium-denuded rings from SHR of the same age. Tissues that failed to increase tension after exposure to phenylephrine were discarded. The presence of endothelium was assessed by testing the relaxation response to acetylcholine ( $10^{-5}$  mol/l). The bath solution was then repeatedly replaced until a stable baseline was restored, and the tension readjusted to 2 g.

In a first series of experiments, a cumulative dose-response curve to ONOO<sup>-</sup> and decomposed ONOO<sup>-</sup> ( $3 \times 10^{-8}$ – $10^{-3}$  mol/l) was tested in phenylephrine-pre-

contracted rings from NAC-treated and untreated WKY rats and SHR (*n* = 6 rats for each group).

In other experiments, the effects of a physiopathological concentration (2 μmol/l) of ONOO<sup>-</sup> on endothelium-dependent and -independent vasodilatation were tested. The effects of cumulative doses of acetylcholine ( $10^{-9}$ – $10^{-5}$  mol/l), sodium nitroprusside (SNP,  $10^{-9}$ – $10^{-5}$  mol/l) and isoproterenol ( $10^{-9}$ – $10^{-5}$  mol/l) on isometric tension of phenylephrine-precontracted aortic rings were evaluated before and after the addition of ONOO<sup>-</sup> or decomposed ONOO<sup>-</sup> (10 μl of a 4 mmol/l ONOO<sup>-</sup> working solution added to a 20 ml organ bath, resulting in a final concentration of 2 μmol/l) to the organ bath. A bolus of ONOO<sup>-</sup> was added 15 min after the first dose-response curve to each drug (acetylcholine, SNP or isoproterenol) and left for 1 min in the organ bath. After the bath solution had been changed, the second dose-response curve to the same drug was repeated. In preliminary experiments, time-response curves to ONOO<sup>-</sup> showed that the maximum concentration of free 3-NT in the organ bath and the lowest concentration of aortic GSH content in WKY rats were reached after incubation for 1 min with a bolus of 2 μmol/l of ONOO<sup>-</sup>.

In a separate set of experiments, free 3-NT dose-response curves (*n* = 4 rat for each group) were performed in endothelium-intact aortic rings ( $10^{-9}$ – $3 \times 10^{-5}$  mol/l) to test its effects on vascular reactivity. We also performed dose-response curves to isoproterenol before and after the addition, for 1 min, of 3-NT at a single concentration of  $2 \times 10^{-7}$  mol/l (maximal concentration reached after the addition of 2 μmol/l ONOO<sup>-</sup> in the bath without aortic rings) to investigate the possible antagonism between the β<sub>2</sub>-adrenergic agonist, isoproterenol, and free 3-NT, which share chemical structure with endogenous catecholamines. All drug concentrations are expressed as final molar concentrations in the organ bath solution. The effects of the drugs were studied in paired rings obtained from the same thoracic aorta.

#### Statistics

All values are expressed as means ± SEM. In each group of experiments, *n* refers to the number of animals from which aortic rings were taken. The statistical comparison of results was done by one-way analysis of variance or by Student's *t*-test. Dose-response curves were analysed in order to compare EC<sub>50</sub> and maximal relaxation response by a curve-fitting analysis program (GraphPad Prism 2.01, GraphPad Inc., USA). EC<sub>50</sub> values were expressed as the negative logarithm of the concentration, which produces 50% of the maximal response to each drug. *P* < 0.05 was considered statistically significant.

## Results

### Systolic and diastolic arterial pressure, heart rate and body weight

Systolic ( $204 \pm 5$  compared with  $133 \pm 7$  mmHg,  $P < 0.01$ ) and diastolic ( $99 \pm 4$  compared with  $69 \pm 4$  mmHg,  $P < 0.01$ ) arterial pressures were significantly greater in 15-week-old SHR than in WKY rats. NAC significantly reduced systolic pressure in SHR ( $13 \pm 3\%$ , SHR-NAC compared with SHR,  $P < 0.01$ ), but not in WKY rats; no effect on diastolic blood pressure was observed in either strain. Heart rate was greater in SHR as compared with WKY rats ( $380 \pm 6$  beats/min compared with  $335 \pm 13$  beats/min,  $P < 0.01$ ), and was significantly reduced by NAC only in SHR ( $346 \pm 7$  beats/min in SHR-NAC;  $P < 0.01$  compared with SHR). Mean body weight was lower in SHR ( $267 \pm 7$  g) than in age-matched WKY rats ( $336 \pm 8$  g,  $P < 0.01$ ), and was not affected by NAC treatment.

### Vascular reactivity

#### Aortic response to ONOO<sup>-</sup>

ONOO<sup>-</sup> induced a relaxation response in endothelium-intact aortic rings, starting from  $10^{-5}$  mol/l, but no effects were seen at lower concentrations (Fig. 1). No effects were found when decomposed ONOO<sup>-</sup> was tested. Furthermore, no difference in dose-response curves to ONOO<sup>-</sup> was observed among aortic rings from NAC-treated and untreated WKY rats and SHR.

#### Relaxation to acetylcholine

The vascular relaxation responses to acetylcholine of endothelium-intact aortic rings from untreated or NAC-

treated WKY rats and SHR before or after exposure to ONOO<sup>-</sup> are illustrated as concentration-response curves in Figure 2, and the EC<sub>50</sub> and maximum relaxation are given in Table 1. The endothelium-dependent relaxation was significantly greater in untreated WKY rats than in untreated SHR, but a significant improvement and even a normalization in maximal relaxation were observed in SHR-NAC. The exposure to ONOO<sup>-</sup> induced a decrease in sensitivity (EC<sub>50</sub>) to acetylcholine in SHR and WKY rats, in addition to a reduction in maximal relaxation only in WKY rats. These effects of ONOO<sup>-</sup> were not altered by NAC treatment in WKY rats, but the NAC treatment prevented the decrease in sensitivity to acetylcholine in SHR.

#### Relaxation to sodium nitroprusside

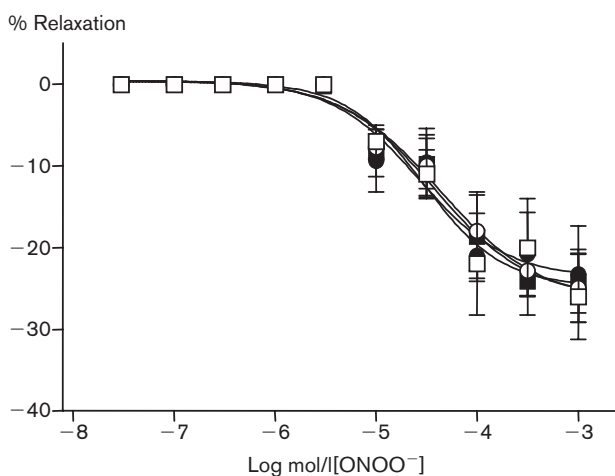
The endothelium-independent nitrovasodilator, SNP, produced a similar concentration-dependent relaxation in endothelium-intact aortic rings of untreated WKY rats and SHR, as shown in Figure 3 and Table 1. NAC treatment did not significantly affect the sensitivity or the maximal relaxation in either strain of rat. Exposure of aortic rings to ONOO<sup>-</sup> caused a rightward shift of the concentration-response curve, with a resulting reduction in sensitivity and in maximal relaxation in WKY rats, the WKY-NAC group, and SHR. A partial but significant protection against the reduction in maximal relaxation induced by ONOO<sup>-</sup> addition was observed only in SHR-NAC rats (Fig. 3 and Table 1).

#### Relaxation to isoproterenol

As depicted in Figure 4 and Table 1, the relaxation in response to isoproterenol did not differ between untreated aortic rings of WKY rats and SHR. Aortic maximal relaxation was increased by NAC treatment in WKY-NAC rats as compared with the WKY rats, whereas it was similar in SHR and in the SHR-NAC group (Fig. 4). After the addition of ONOO<sup>-</sup> to the bath chamber, rightward shifts of the dose-response curves were observed in both strains of rat (Fig. 4 and Table 1). The SHR-NAC group showed an improvement in sensitivity to isoproterenol after exposure to ONOO<sup>-</sup> as compared with SHR. The addition of decomposed ONOO<sup>-</sup> to the organ bath did not alter vascular relaxing responses to acetylcholine, SNP and isoproterenol (data not shown).

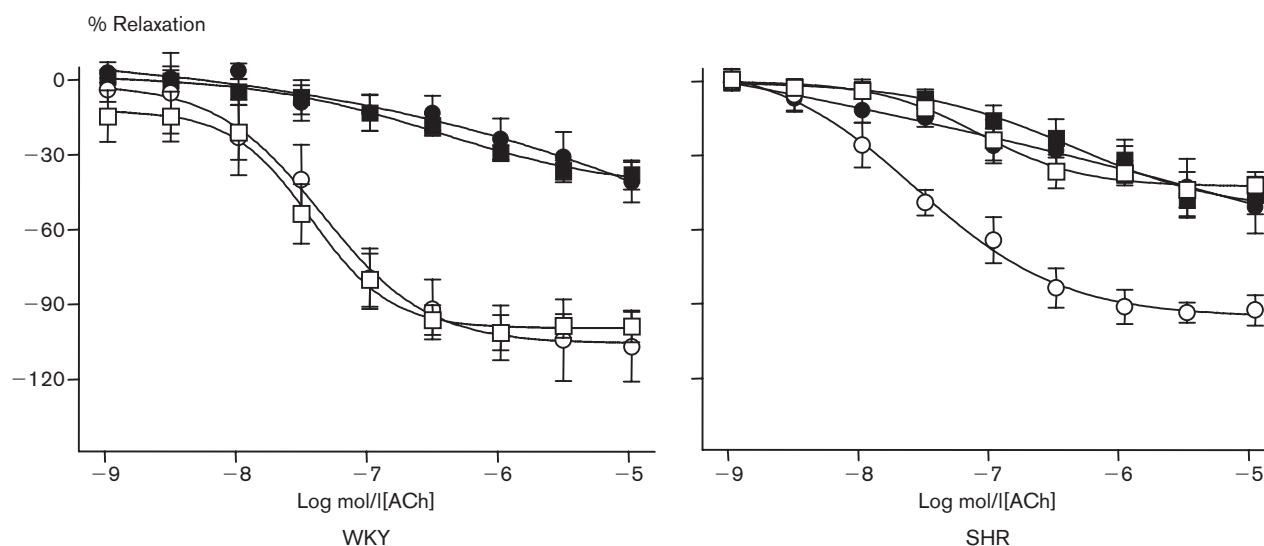
Free 3-NT, tested in the concentration range  $10^{-9}$ – $3 \times 10^{-5}$  mol/l, neither contracted nor relaxed phenylephrine-precontracted aortic rings from all groups. In addition, no difference in dose-response curves to isoproterenol were found before and after a 1 min addition of free 3-NT, thus excluding an antagonistic effect of free 3-NT on isoproterenol-mediated relaxation (data not shown).

Fig. 1



Dose-response curves to peroxynitrite (ONOO<sup>-</sup>) in endothelium-intact aortic rings from *N*-acetylcysteine (NAC)-treated Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) ( $n = 6$  for each group: ○ WKY-NAC; ● SHR-NAC) and untreated WKY rats (□;  $n = 6$ ) and untreated SHR (■;  $n = 6$ ). Data are expressed as mean  $\pm$  SEM.

Fig. 2



Dose-response curves showing endothelium-dependent relaxation responses to acetylcholine (ACh) before and after exposure to peroxynitrite ( $\text{ONOO}^-$ ) ( $2 \mu\text{mol/l}$ ) in endothelium-intact aortic rings. Left panel: *N*-acetylcysteine (NAC)-treated Wistar-Kyoto (WKY) rats (WKY-NAC,  $n = 12$ : before  $\text{ONOO}^-$ ,  $\square$ ; after  $\text{ONOO}^-$ ,  $\bullet$ ) and untreated WKY rats (WKY,  $n = 11$ : before  $\text{ONOO}^-$ ,  $\square$ ; after  $\text{ONOO}^-$ ,  $\bullet$ ). Right panel: NAC-treated spontaneously hypertensive rats (SHR-NAC,  $n = 11$ : before  $\text{ONOO}^-$ ,  $\square$ ; after  $\text{ONOO}^-$ ,  $\bullet$ ) and untreated SHR (SHR,  $n = 11$ : before  $\text{ONOO}^-$ ,  $\square$ ; after  $\text{ONOO}^-$ ,  $\bullet$ ). Data are expressed as mean  $\pm$  SEM.

#### GSH and GSSG aortic content before and after the addition of $\text{ONOO}^-$

GSH concentrations were similar in aortic homogenates from SHR ( $n = 7$ ) and WKY rats ( $n = 7$ ); a greater reduction in GSH aortic content was observed after  $\text{ONOO}^-$  exposure in SHR (36%) than in WKY rats (24%). NAC treatment, which doubled GSH aortic content in WKY rats but increased GSH only by 55% in SHR, did, however, prevent GSH depletion after the addition of  $\text{ONOO}^-$  in both groups (Table 2). GSSG aortic content was significantly greater in SHR than in WKY rats; the addition of  $\text{ONOO}^-$  induced a further increase of 3.7 times the concentrations in SHR and also increased the concentrations by 3.9 times in WKY rats, but those increases were partially prevented in NAC-treated groups. GSSG/GSH ratios, which were greater in SHR than in WKY rats before and after exposure to  $\text{ONOO}^-$ , were significantly reduced in NAC-treated groups, and even more so in SHR-NAC than in WKY NAC rats (Fig. 5).

#### Malondialdehyde aortic content before and after the addition of $\text{ONOO}^-$

As depicted in Figure 6, malondialdehyde concentrations were 35% greater in aortic homogenates from SHR ( $n = 7$ ) compared with those from WKY rats ( $P = 0.02$ ,  $n = 7$ ). The addition of  $\text{ONOO}^-$  significantly increased aortic malondialdehyde content in both strains, but especially in SHR (by 46% in SHR and 21% in WKY rats; Fig. 6). Chronic NAC treatment

reduced malondialdehyde aortic content concentrations before the addition of  $\text{ONOO}^-$  in both strains (by 21% in SHR, and 16% in WKY rats). In addition, NAC partially prevented the increase in malondialdehyde after exposure to  $\text{ONOO}^-$  both in SHR (by 25% in NAC-treated and 46% in untreated SHR) and in WKY rats (by 8% in NAC-treated WKY rats and 21% in untreated WKY rats; Fig. 6).

#### Free L-tyrosine nitration under basal conditions and after the addition of $\text{ONOO}^-$

As shown in Figure 7, no differences in free 3-NT concentrations were measured between WKY rats, SHR, and NAC-treated groups before exposure to  $\text{ONOO}^-$ . After the addition of  $\text{ONOO}^-$  to the organ bath in the absence of aortic rings, 3-NT concentrations reached  $189.9 \pm 2.9 \text{ nmol/l}$ . In the presence of aortic rings, generation of 3-NT was reduced by  $45 \pm 2\%$  in WKY rats and by  $46 \pm 5\%$  in the WKY-NAC group, but only by  $27 \pm 2\%$  in SHR, and  $38 \pm 4\%$  in the SHR-NAC group, thus suggesting a reduced capacity of SHR aorta to inactivate  $\text{ONOO}^-$  or to antagonize the nitration of L-tyrosine in the bath, as compared with untreated WKY rats and NAC-treated groups. Decomposed  $\text{ONOO}^-$  was ineffective in nitrating L-tyrosine (data not shown).

#### Discussion

Two weeks of oral treatment with NAC decreased the *in vivo* systolic arterial pressure only in the SHR,

**Table 1** Effects of N-acetylcysteine (NAC) treatment on sensitivity (EC<sub>50</sub>, -log) and maximal relaxation (Max Rel; expressed as percentage of the contractile response to phenylephrine on basal tension) to acetylcholine, sodium nitroprusside and isoproterenol in endothelium-intact aortic rings of 15-week-old treated Wistar-Kyoto rats and spontaneously hypertensive rats (WKY-NAC, SHR-NAC), and untreated WKY and SHR before and after exposure to ONOO<sup>-</sup>

Group	Acetylcholine						Sodium nitroprusside						Isoproterenol					
	Basal		After ONOO <sup>-</sup>		Basal		After ONOO <sup>-</sup>		Basal		After ONOO <sup>-</sup>		Basal		After ONOO <sup>-</sup>			
	EC <sub>50</sub> (-log)	Max rel (%)	EC <sub>50</sub> (-log)	Max rel (%)	EC <sub>50</sub> (-log)	Max rel (%)	EC <sub>50</sub> (-log)	Max rel (%)	EC <sub>50</sub> (-log)	Max rel (%)	EC <sub>50</sub> (-log)	Max rel (%)	EC <sub>50</sub> (-log)	Max rel (%)	EC <sub>50</sub> (-log)	Max rel (%)		
WKY	7.44 ± 0.04	105 ± 7	6.47 ± 0.13**	42 ± 6**	7.67 ± 0.06	166 ± 14	6.62 ± 0.05**	81 ± 6**	7.34 ± 0.09	114 ± 7	6.32 ± 0.10**	68 ± 8**	7.34 ± 0.09	114 ± 7	6.32 ± 0.10**	68 ± 8**		
WKY-NAC	7.37 ± 0.05	111 ± 14	5.73 ± 0.20**	45 ± 8**	7.80 ± 0.07	174 ± 16	6.44 ± 0.07**	81 ± 11**	7.29 ± 0.07	155 ± 7††	6.04 ± 0.22**	77 ± 9**	7.29 ± 0.07	155 ± 7††	6.04 ± 0.22**	77 ± 9**		
SHR	7.11 ± 0.08	46 ± 7‡	6.48 ± 0.18*	51 ± 7	7.80 ± 0.16	139 ± 17	6.72 ± 0.07**	66 ± 7**	6.98 ± 0.11	118 ± 9	5.76 ± 0.07**	72 ± 7**	6.98 ± 0.11	118 ± 9	5.76 ± 0.07**	72 ± 7**		
SHR-NAC	7.49 ± 0.09	96 ± 4††	6.91 ± 0.32	54 ± 10*	7.94 ± 0.21	145 ± 16	6.82 ± 0.05**	114 ± 14††	6.86 ± 0.08	112 ± 12	5.87 ± 0.12**†	75 ± 8**	6.86 ± 0.08	112 ± 12	5.87 ± 0.12**†	75 ± 8**		

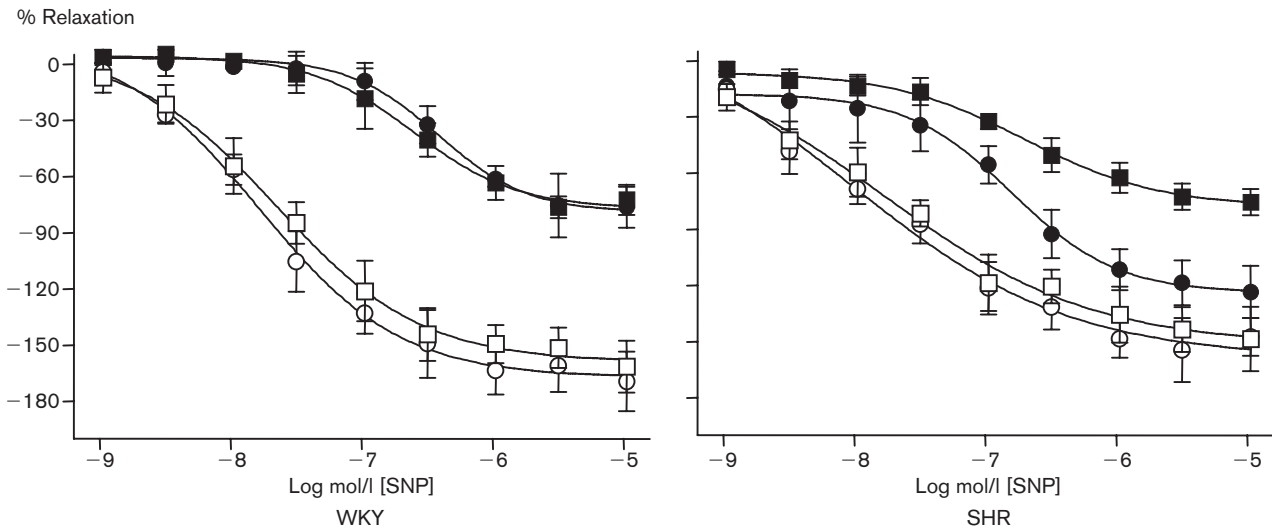
\* P < 0.05, \*\* P < 0.01 compared with basal value; † P < 0.05, †† P < 0.01 compared with untreated; ‡ P < 0.01 compared with WKY.

whereas no effects on diastolic blood pressure were observed, suggesting an inefficacy on resistance vessels tonus. In a recent study, NAC was shown to improve the endothelium-dependent dilation of isolated mesenteric resistance arteries [17]; however, in an *in vivo* situation additional mechanisms independent of the endothelium, including neural or humoral factors, are involved in the control of peripheral vascular resistance. Moreover, the present study demonstrated that chronic oral treatment with NAC reversed the impairment of endothelium-dependent aortic relaxation in response to acetylcholine in SHR as compared with normotensive WKY rats. An *in vivo* improvement in acetylcholine-induced vasodilatation after an acute intravenous administration of NAC has also been reported [18], but no differences between WKY rats and SHR were observed.

The protective effect of NAC on the endothelium-dependent relaxation in SHR may depend on a reduction in the aortic GSSG/GSH ratio, as GSH has been demonstrated to be necessary for basal and stimulated production of nitric oxide by endothelial cells [19]. SHR, in fact, showed a greater GSSG/GSH aortic content ratio compared with WKY rats, and this difference was mainly due to an increased GSSG content in SHR, because GSH content was similar in both strains. These findings are in line with the results observed in other tissues of 16-week-old SHR, showing an altered turnover rate of the GSH redox cycle, with reduced activity of GSH peroxidase and GSSG reductase and an increase in the intracellular content of GSSG [20–22]. In our study, the effects of NAC on basal aortic GSSG/GSH balance, showing an increase in GSH without a parallel decrease in GSSG concentrations, may depend on the promotion of GSH synthesis via its capacity to increase the intracellular concentration of L-cysteine, which is the rate-limiting component in GSH synthesis [23]. Under our experimental conditions, the promotion of GSH synthesis appeared to be the main effect of NAC on GSSG/GSH balance, even if other actions of NAC have been demonstrated. In fact, additional protective effects of NAC have been related to its direct scavenging properties and to the up-regulation of antioxidant systems including GSH peroxidase and GSSG reductase [24,25].

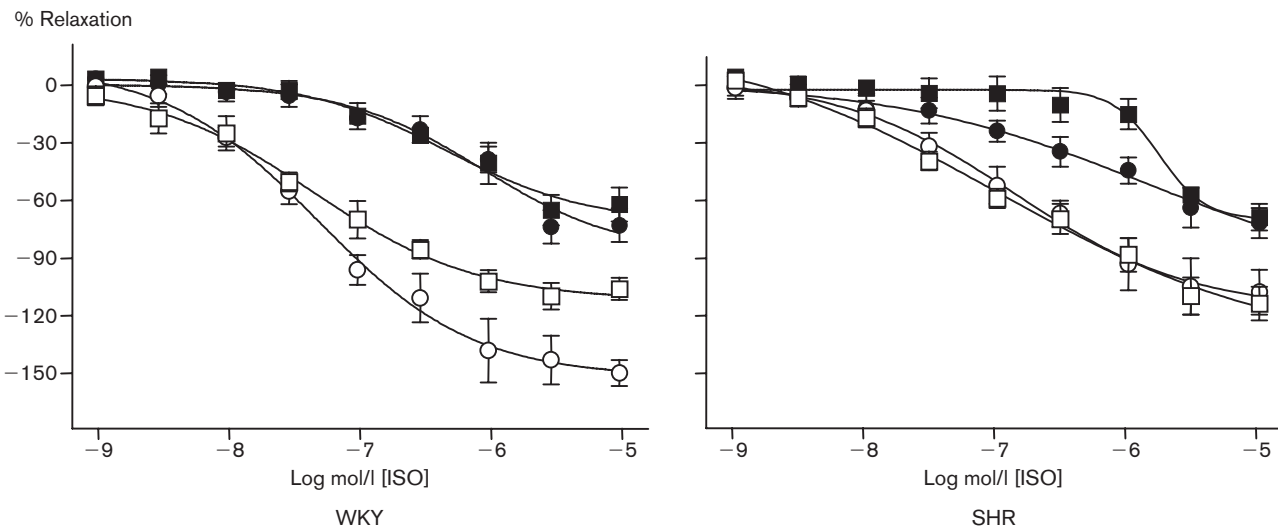
In this study, NAC also exerted its antioxidant effects by reducing aortic lipid peroxidation in both strains of rat, as indicated by the lower concentrations of malondialdehyde before and after ONOO<sup>-</sup>. SHR, as previously reported by others but in different tissues [22,26], showed greater levels of lipid peroxidation in aortas; they may therefore have benefited from the NAC-induced reduction of malondialdehyde, as this moiety has recently been demonstrated to impair

Fig. 3



Dose-response curves showing endothelium-independent relaxation responses to sodium nitroprusside (SNP) before and after exposure to peroxynitrite (ONOO<sup>-</sup>) (2  $\mu$ mol/l) in endothelium-intact aortic rings. Left panel: *N*-acetylcysteine (NAC)-treated Wistar-Kyoto (WKY) rats (WKY-NAC,  $n = 12$ : before ONOO<sup>-</sup>,  $\circ$ ; after ONOO<sup>-</sup>,  $\bullet$ ) and untreated WKY rats (WKY,  $n = 11$ : before ONOO<sup>-</sup>,  $\square$ ; after ONOO<sup>-</sup>,  $\blacksquare$ ). Right panel: NAC-treated spontaneously hypertensive rats (SHR-NAC,  $n = 11$ : before ONOO<sup>-</sup>,  $\circ$ ; after ONOO<sup>-</sup>,  $\bullet$ ) and untreated SHR (SHR,  $n = 10$ : before ONOO<sup>-</sup>,  $\square$ ; after ONOO<sup>-</sup>,  $\blacksquare$ ). Data are expressed as mean  $\pm$  SEM.

Fig. 4



Dose-response curves showing relaxing responses to isoproterenol (ISO) before and after exposure to peroxynitrite (ONOO<sup>-</sup>) (2  $\mu$ mol/l) in endothelium-intact aortic rings. Left panel: *N*-acetylcysteine (NAC)-treated Wistar-Kyoto (WKY) rats (WKY-NAC,  $n = 12$ : before ONOO<sup>-</sup>,  $\circ$ ; after ONOO<sup>-</sup>,  $\bullet$ ) and untreated WKY rats (WKY,  $n = 12$ : before ONOO<sup>-</sup>,  $\square$ ; after ONOO<sup>-</sup>,  $\blacksquare$ ). Right panel: NAC-treated spontaneously hypertensive rats (SHR-NAC,  $n = 11$ : before ONOO<sup>-</sup>,  $\circ$ ; after ONOO<sup>-</sup>,  $\bullet$ ) and untreated SHR (SHR,  $n = 11$ : before ONOO<sup>-</sup>,  $\square$ ; after ONOO<sup>-</sup>,  $\blacksquare$ ). Data are expressed as mean  $\pm$  SEM.

endothelium-dependent relaxation by increasing superoxide anion generation [27].

In this study, we also observed that both SNP- and

isoproterenol-mediated aortic relaxations did not differ between WKY rats and SHR, whereas the isoproterenol-mediated relaxation response was potentiated in NAC-treated WKY rats. Such a finding could suggest a

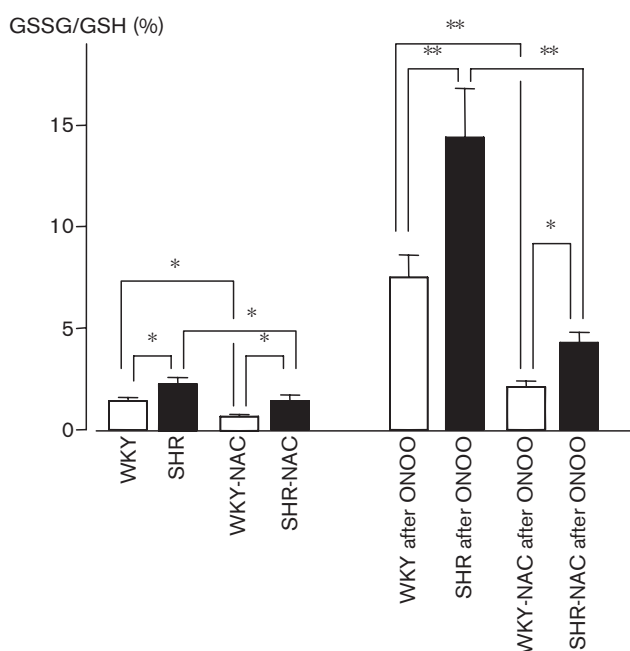


**Table 2** Reduced (GSH) and oxidized (GSSG) glutathione content in aortic homogenates from *N*-acetylcysteine (NAC)-treated (WKY-NAC, SHR-NAC) and control Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) before and after exposure to ONOO<sup>-</sup> (2 μmol/l)

Group	Aortic GSH (nmol/mg protein)		Aortic GSSG (pmol/mg protein)	
	Before ONOO <sup>-</sup>	After ONOO <sup>-</sup>	Before ONOO <sup>-</sup>	After ONOO <sup>-</sup>
WKY	7.5 ± 0.9	5.7 ± 0.7	99 ± 13‡	389 ± 22
WKY-NAC	15.3 ± 1.2**	14.5 ± 1.1**	114 ± 9‡	297 ± 24**†
SHR	7.3 ± 0.8‡	4.7 ± 0.7†	161 ± 16¶§‡	603 ± 52¶§†
SHR-NAC	11.4 ± 1.2**¶	10.3 ± 0.8**§	144 ± 26‡	426 ± 33

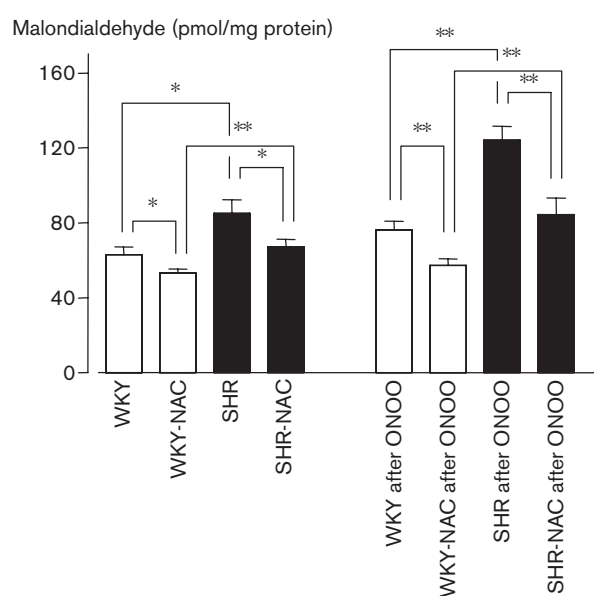
¶ *P* < 0.05 compared with WKY; § *P* < 0.05 compared with WKY-NAC; † *P* < 0.05 compared with SHR-NAC; ‡ *P* < 0.05 compared with after ONOO<sup>-</sup> exposure, \*\* *P* < 0.01 compared with untreated.

**Fig. 5**



Bar graph showing the effects of peroxynitrite (ONOO<sup>-</sup>) (2 μmol/l) on oxidized/reduced (GSSG/GSH) glutathione ratio content in homogenates of aortic rings from *N*-acetylcysteine (NAC)-treated and untreated Wistar-Kyoto (WKY) rats (WKY-NAC and WKY rats, open bars), and NAC-treated and untreated spontaneously hypertensive rats (SHR-NAC and SHR, solid bars); *n* = 7 rats for each group. Data are expressed as mean ± SEM. \* *P* < 0.05; \*\* *P* < 0.01.

**Fig. 6**



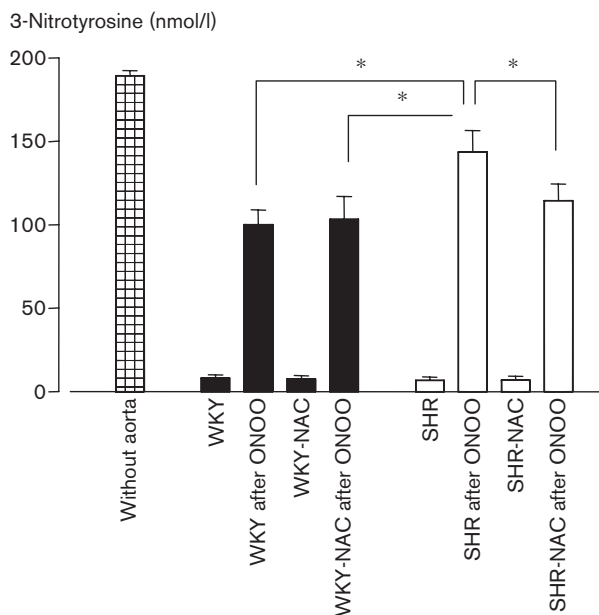
Bar graph showing the effects of peroxynitrite (ONOO<sup>-</sup>) (2 μmol/l) on malondialdehyde content in homogenates of aortas from *N*-acetylcysteine (NAC)-treated and untreated Wistar-Kyoto (WKY) rats (WKY-NAC, *n* = 7 and WKY rats, *n* = 8, open bars), and NAC-treated and untreated spontaneously hypertensive rats (SHR-NAC, *n* = 8 and SHR, *n* = 9, solid bars). Data are expressed as mean ± SEM. \* *P* < 0.05; \*\* *P* < 0.01.

role for free radicals in the modulation of the β-adrenoceptor response under normotensive conditions. Persad *et al.* [28] demonstrated the involvement of free radicals, particularly of superoxide anion, in the alteration of cardiac β-adrenoceptor signal transduction and its reversibility by superoxide dismutase administration.

The mechanisms by which isoproterenol induces vasorelaxation are varied and pathways have been proposed other than the activation of adenylyl cyclase in smooth muscle cells, which results in a reduction of intracellu-

lar Ca<sup>2+</sup> concentrations through the activation of cAMP-dependent protein kinase [29]. In fact, endothelium-derived factors are also involved in isoproterenol-mediated vasorelaxation. It has been reported that activation of the β-adrenoceptor induces the release of nitric oxide from vascular endothelium, leading to the stimulation of cGMP formation, which inhibits the degradation of cAMP [30,31]. In addition, the endothelium-derived hyperpolarizing factor appears to be involved in the hyperpolarization of vascular smooth muscle through either Ca<sup>2+</sup>-activated or ATP-sensitive K<sup>+</sup> channel pathways that seem to be fundamental to isoproterenol-mediated aortic relaxation in rats [32–34].

Fig. 7



Bar graph showing the effects of the addition of peroxynitrite ( $\text{ONOO}^-$ ) (2  $\mu\text{mol/l}$ ) on the formation of free 3-nitrotyrosine in an organ bath filled with L-tyrosine-added Krebs-Henseleit buffer in the absence (squared bars) or the presence of aortic ring from *N*-acetylcysteine (NAC)-treated and untreated Wistar-Kyoto (WKY) rats (WKY-NAC and WKY rats, open bars), and NAC-treated and untreated spontaneously hypertensive rats (SHR-NAC and SHR, solid bars);  $n = 10$  rats per group. Data are expressed as mean  $\pm$  SEM. \* $P < 0.05$ .

Some of these mechanisms could be affected by the generation of free radicals [28,35], which may explain why treatment with NAC improved aortic relaxation in response to isoproterenol in WKY rats.

In the present study, basal release of  $\text{ONOO}^-$ , measured by formation of free 3-NT in the organ bath, was similar in the aortas of both strains, and was unaffected by NAC treatment. However, a difference in  $\text{ONOO}^-$  release between SHR and WKY rats cannot be excluded, because in this study we did not assess protein-bound 3-NT expression, which was recently found to be increased in aortas from rats made hypertensive by aortic banding [36]. We observed that, in the presence of the aortic ring, the addition of  $\text{ONOO}^-$  to the organ bath induced a smaller increase in free 3-NT. Such a difference may depend on the ability of vessels to scavenge  $\text{ONOO}^-$ ; hence, the reduction in free tyrosine nitration in the bath may represent an index of the antioxidant capacity of the aortic tissue. After the addition of  $\text{ONOO}^-$ , a greater extent of free 3-NT was found in the bath containing aortic rings from untreated SHR than in those from the other groups. This may suggest an increased vulnerability of SHR aorta to  $\text{ONOO}^-$ -mediated cellular damage, presumably depen-

dent on a deficient antioxidant defence, including the observed greater GSSG/GSH intracellular ratio and lipid peroxidation, the reduced activity of cytoplasmic and mitochondrial superoxide dismutases and catalase [37], and vitamin E concentrations [38]. NAC treatment significantly reduced 3-NT concentrations in the SHR aortic ring bath, by increasing aortic tissue-scavenging properties against  $\text{ONOO}^-$ .

In vascular reactivity experiments we have shown that  $\text{ONOO}^-$  induced a concentration-dependent relaxation in endothelium-intact aortic rings. The vasodilator capacities of  $\text{ONOO}^-$  have already been reported in isolated vessels from the conductance vascular bed [39,40] and *in vivo* [41], but until now no data have been available on the effects of  $\text{ONOO}^-$  in arteries exposed to sustained hypertension. Chronic hypertension or NAC treatment, or both, does not modify the aortic vascular ring response to  $\text{ONOO}^-$  compared with that in normotensive WKY rats. The mechanisms proposed for  $\text{ONOO}^-$ -mediated vasorelaxation, such as the increase in cGMP by the activation of guanylyl cyclase [42], or hyperpolarization of vascular smooth muscle cells by the activation of ATP-sensitive potassium channels [41], appear to be unaffected in aortas from hypertensive rats and uninfluenced by an increased availability of cellular thiol. In the present study we have demonstrated that greater concentrations of  $\text{ONOO}^-$  (tens to hundreds of micromoles) induced aortic relaxation in all groups, whereas no effects were detectable at lower concentrations. A matter of great debate is the physiological plasma concentration of  $\text{ONOO}^-$  and whether or not *in vivo*  $\text{ONOO}^-$  concentrations could reach the high micromolar to millimolar values. Kelm *et al.* [43] have shown that the circulating physiological concentration of nitric oxide may be in the low nanomolar range, but can increase by two or three orders of magnitude in physiopathological conditions in which inducible nitric oxide synthase is activated [44]. As nitric oxide must be equimolar to superoxide in order to generate  $\text{ONOO}^-$  [45], the effective concentration of  $\text{ONOO}^-$  would remain in the nanomolar or very low micromolar range. Therefore,  $\text{ONOO}^-$  concentrations that induced vasorelaxant effects in our aortic rings are unlikely to occur in the *in vivo* situation, even when inducible nitric oxide synthase is strongly activated. That is the reason why a concentration in the low micromolar range (2  $\mu\text{mol/l}$ ) was used in our experiments on the effects of  $\text{ONOO}^-$  on endothelium-dependent and independent vasodilatation.

Exposure to  $\text{ONOO}^-$  at a concentration in the physiopathological range affected both endothelium-dependent and -independent aortic relaxation in the normotensive and hypertensive strains of rats. A similar pattern of effects of  $\text{ONOO}^-$  in the coronary micro-

circulation of normotensive rats has been described by Villa *et al.* [46]. The maximal relaxation of aortic rings in WKY rats and the sensitivity to acetylcholine in SHR were impaired after exposure to ONOO<sup>-</sup>. The decreased sensitivity to acetylcholine after ONOO<sup>-</sup> was prevented in NAC-treated SHR. ONOO<sup>-</sup> may directly affect endothelium-dependent relaxation to acetylcholine at various sites, including the endothelial muscarinic receptor, the post-receptor signalling pathway leading to the activation of intracellular endothelial nitric oxide synthase. Recently, inhibitory effects of ONOO<sup>-</sup> have been found on the activation of the M3 muscarinic receptor [47], on intracellular calcium signalling of vascular endothelial cells [48], and on endothelial nitric oxide synthase [49] – all potentially leading to a reduced acetylcholine-mediated relaxation. In contrast, Tarpey *et al.* [42] reported that ONOO<sup>-</sup> at high concentrations may facilitate endothelium-dependent relaxation by increasing endothelium-dependent cGMP synthesis through a glutathione-dependent mechanism of stimulation of guanylyl cyclase. In the present study, the greater concentration of aortic malondialdehyde after the addition of ONOO<sup>-</sup>, observed in both strains of rat but more pronounced in SHR, may be involved in the impairment of the acetylcholine-mediated relaxing response [27]. An impaired aortic relaxation response to acetylcholine after exposure to ONOO<sup>-</sup> can also be dependent on its inhibitory effect, even at low concentration, on prostacyclin synthase activity, resulting in a prevalence of endothelium-derived contracting factors such as cyclic endoperoxide (PGH<sub>2</sub>) or thromboxane A<sub>2</sub> released by aortas in response to acetylcholine [50]. It has also recently been reported that free 3-NT may contribute directly to selective vascular endothelial dysfunction by the activation of pro-apoptotic pathways in endothelial cells [51].

The endothelium-independent relaxation in response to SNP, which was similar in NAC-treated and untreated groups, was also markedly impaired by exposure to ONOO<sup>-</sup> in both strains of rat, with a partial protection provided by NAC only in SHR. Our results agree with those of Villa *et al.* [46], which showed a decrease in endothelium-independent relaxation in isolated heart after perfusion with a low concentration of ONOO<sup>-</sup>. ONOO<sup>-</sup> is known to induce the conversion of xanthine dehydrogenase to the oxidase form that generates greater amounts of superoxide [52] leading – through reaction with nitric oxide released from SNP – to the production of ONOO<sup>-</sup>, which is known to be less potent than exogenous nitric oxide in stimulating guanylyl cyclase [42]. However, the same study [42] demonstrated that ONOO<sup>-</sup>, at a concentration of 100 μmol/l, doubled the SNP-induced synthesis of cGMP in smooth muscle cell culture. Although a thiol-dependent mechanism has been demonstrated in the activation of smooth muscle guanylyl cyclase by

ONOO<sup>-</sup> in vascular tissues [53], the mechanism of the protective effect of NAC on SNP-mediated aortic relaxation in hypertensive, but not in normotensive, rats was not investigated in the present study.

The relaxation in response to isoproterenol was also markedly attenuated after exposure to ONOO<sup>-</sup>. It has recently been demonstrated by Kooy's group [54] that ONOO<sup>-</sup> attenuates β-adrenoceptor responses to isoproterenol *in vivo*, probably through its oxidizing and nitrosating properties, leading to an alteration in receptor structure, which results in decreased affinity and response. The same authors suggested that the increase in free 3-NT concentrations may be involved in the impaired aortic relaxation to isoproterenol after exposure to ONOO<sup>-</sup>, through a variable antagonistic activity on the β-adrenoceptor response. However, in this study we did not observe any antagonistic effects of free 3-NT on isoproterenol-mediated relaxation.

In summary, our data suggest that chronic NAC treatment induces a reduction in systolic arterial pressure, and improves endothelium-dependent aortic relaxation in SHR. Furthermore, NAC protects SHR aortas from impaired relaxation responses to SNP and isoproterenol after exposure to ONOO<sup>-</sup>. These observations, along with the greater GSSG/GSH aortic content ratio, the increased malondialdehyde concentrations, and the reduced capacity to scavenge ONOO<sup>-</sup>, indicate that SHR aortic vessels are more vulnerable to endothelial damage and to endothelium-independent alterations in vascular reactivity upon exposure to physiopathological concentrations of ONOO<sup>-</sup>. Although the exact clinical relevance of those observations is not yet known, these findings nevertheless suggest that NAC treatment may be beneficial in the management of altered aortic vascular reactivity in the SHR model of hypertension.

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