N-Acetylcysteine Improves Nitric Oxide and α-Adrenergic Pathways in Mesenteric Beds of Spontaneously Hypertensive Rats

Hélène Girouard, Chantal Chulak, Lingyun Wu, Mireille Lejossec, and Jacques de Champlain

Background: The aim of this study was to assess the effects of *N*-acetylcysteine (NAC) on nitric oxide and adrenergic pathways in mesenteric artery from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY).

Methods: Rats were treated with 4 $g \cdot kg^{-1} \cdot day^{-1}$ of NAC during 4 weeks or mesenteric beds were treated with 10 mmol/L of NAC during 20 min.

Results: In conscious rats, the NAC treatment produced a significant reduction of mean arterial pressure (MAP) and heart rate in SHR ($P < .001$). N^{ω}-nitro-L-arginine methyl ester (L-NAME) caused a MAP increase in NAC-treated SHR of magnitude similar to that in WKY, which was significantly higher than that observed in control untreated SHR ($P < .05$). Chronic treatments with NAC improved the maximal relaxation of mesenteric arteries to A23187 in SHR ($P < .001$). Acute NAC treatment in vitro induced a vasodilation in Phe preconstricted arteries ($P < .001$) that was stronger in SHR than in WKY ($P < .05$) and was not abolished by L-NAME. The vasoconstrictory response and increases in inositol phosphate production induced by superoxide anion were attenuated by NAC treatment through its superoxide scavenging properties. In contrast, chronic and acute NAC treatments did not alter the vasodilatory response to β-adrenergic receptor stimulation.

Conclusions: The increase in NO-mediated vasodilator tone and the possible decrease in adrenergic vasoconstriction induced by NAC treatment in SHR could explain the hypotensive effect of NAC in this model of hypertension. Am J Hypertens 2003;16:577–584 © 2003 American Journal of Hypertension, Ltd.

Key Words: *N*-acetylcysteine, adrenergic α-agonists, nitric oxide synthase, free radicals, antioxidants.

The spontaneously hypertensive rat (SHR) experimental model of hypertension is characterized by an increased α-adrenergic–induced vasoconstriction,[1] as well as by a decreased β-adrenergic–induced vasorelaxation in resistance arteries[2] and a decreased muscarinic induced vasorelaxation in aorta[3] and resistance arteries.[4]

Acute or chronic treatments with *N*-acetylcysteine (NAC), a thiol-containing compound and a powerful antioxidant, were found to increase the endothelium-dependent relaxation in mesenteric arteries[5] and in aorta[6] as well as to enhance the hypotensive effect of acetylcholine (Ach) in vivo in normotensive and in SHR through a nitric oxide (NO)–dependent mechanism.[7] Moreover, it has also been demonstrated that NAC can potentiate the antihypertensive response of angiotensin converting enzyme inhibitors in SHR by a NO-dependent mechanism.[8]

However, the potential effect of NAC on adrenergic pathways has never been studied. Because α- and β-adrenergic pathways could be largely influenced by a NO-dependent mechanism[9] and altered by free radicals,[10,11] we hypothetized that NAC could also modify the vasoreactivity to α- and β-adrenergic agonists. Thus, the aim of the present study was to evaluate whether α- and β-adrenergic functions are modified after NAC treatment in SHR and control Wistar-Kyoto rats (WKY) and whether those modifications are NO dependent.

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From the Research Group on Autonomic Nervous System, Department of Physiology, Faculty of Medicine, University of Montréal, Montréal, Québec, Canada.

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Address correspondence and reprint requests to Dr. Jacques de Champlain, Department of Physiology, Faculty of Medicine, Université de Montréal, C.P. 6128, Succ. Centre-ville, Montréal, Québec, Canada, H3C 3J7; e-mail: grsna@ere.umontreal.ca

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Table 1. Body weight, mean arterial pressure, and heart rate in control and NAC-treated WKY and SHR

Group	Body Weight (g)	MAP (mm Hg)	Heart Rate (beats/min)
WKY	296.5 ± 5.3	102.9 ± 3.4	314.0 ± 24.1
WKY-NAC	306.3 ± 6.8	102.6 ± 2.2	326.1 ± 4.2
SHR	315.4 ± 5.2	151.5 ± 2.5*	380.3 ± 6.4†
SHR-NAC	319.1 ± 4.1	131.1 ± 5.4*‡	328.8 ± 11.7§

MAP = mean arterial pressure; NAC = *N*-acetylcysteine; SHR = spontaneously hypertensive rats; WKY = Wistar-Kyoto rats.
Values are mean ± SEM., $n = 8–12$.
* $P < .001$; † $P < .01$ SHR *v* WKY; ‡ $P < .001$; § $P < .05$; NAC-treated *v* untreated.
Statistical analysis: one-way ANOVA in conjunction with Bonferroni correction.

Methods

Animals and Treatment

Both SHR and normotensive WKY (11 weeks old) were obtained from Harlan Laboratories (Indianapolis, IN). In studies performed to assess the effect of free radicals on vasoreactivity, the inositol phosphate (IP) formation and the antioxidant effect of NAC, Sprague-Dawley rats were used to study the effect independently of the hypertensive state. The rats were treated and maintained in accordance with Canadian Council on Animal Care guidelines and the study was approved by the local Institutional Animal Ethics Committee. The rats were housed under conditions of constant temperature and humidity, exposed to a 12-h light/dark cycle, and given free access to standard laboratory rat chow (Basal Purified Diet 5755C, Purina Mills Inc., St. Louis, MO) and drinking water. After a few days of acclimatization, rats were randomly divided into control groups and NAC-treated groups receiving 4 $g \cdot kg^{-1}$ of body weight per day of NAC during 4 weeks in their drinking water. Each day at 6 PM NAC was dissolved into the drinking water.

Determination of BP

After 4 weeks of NAC treatment, mean arterial pressure (MAP) and heart rate (HR) were measured in chronically cannulated conscious and unrestrained animals under resting conditions as previously described.[12] When MAP had reached stable basal levels, after at least 20 min of continuous recording, basal values were measured. At the end of the baseline period, a bolus injection of N^{ω}-nitro-L-arginine methyl ester (L-NAME) was administered every 10 min at increasing concentrations (0.5 to 20 $mg \cdot kg^{-1}$) through the left femoral venous catheter. No differences in daily intake and body weight were observed among groups.

Mesenteric Arterial Bed Preparation

Experiments were performed on 15-week-old rats as previously described.[12] The mesenteric bed was continuously perfused with 60 mmol/L KCl, which produced about 50% of maximal contraction in SHR and WKY. The presence of a functional endothelium was assessed by the observation of a vasodilator action of Ach (10 μmol/L). The mesenteric arterial bed preparation was then perfused with increasing and cumulative concentrations of Ach, A23187, sodium nitroprusside (SNP), isoproterenol (Iso), and phenylephrine (Phe) at 5-min intervals. In chronic studies, dose-response curves were constricted for three to four drugs in each mesenteric artery, and the order of the addition was randomized. The mesenteric beds were perfused during 1 h between each dose-response curves with Krebs bicarbonate solution containing 60 mmol/L KCl or with Krebs bicarbonate solution only before Phe-induced vasoconstriction. No prior exposure affected the subsequent response to the drug. However, in acute studies, only one drug was tested 1 h before or during NAC treatment. NAC (10 mmol/L) was added 20 min before the beginning of the dose-response curves. This concentration and time of preincubation for NAC correspond to its maximal vasorelaxing effect on hypoxanthine-xanthine oxidase and phenylephrine-induced vasoconstriction. Chronic or acute NAC treatments did not change the constriction response of mesenteric beds to 60 mmol/L KCl. L-NAME (100 μmol/L) was added to the perfusate 20 min before the induction of near 70 mm Hg increase in perfusion pressure by Phe (0.3 to 3 μmol/L) to assess a possible contribution of NOS pathway in NAC induced vasorelaxation. When the increase perfusion had reached a plateau, NAC (10 mmol/L) was added for 20 min. L-NAME did not change the baseline differently between the two strains.

In Sprague-Dawley rats, the effects of increasing and cumulative concentrations of the free radical generating system hypoxanthine-xanthine oxidase was assessed at 30-min intervals in mesenteric beds with or without endothelium. Mesenteric beds were preconstricted with 60 mmol/L KCl. The endothelium was removed by 5 min of perfusion with distilled water.

Cell Culture

Single smooth muscle cells (SMCs) from mesenteric arteries were prepared as described previously.[13] Cells between passages 2 and 8 were seeded into 132-mm, six-well multidishes and used for IP. There was no significant difference in the superoxide anion–induced increase in the formation of IP in cultured SMCs among passages 2 to 8.

Table 2. Effects of *N*-acetylcysteine on responses to vasorelaxant drugs in mesenteric bed of WKY and SHR

	Ach		A23187		SNP		ISO	
Group	ED_{50} (−log)	Max Rel (−%)	ED_{50} (−log)	Max Rel (−%)	ED_{50} (−log)	Max Rel (−%)	ED_{50} (−log)	Max Rel (−%)
WKY	7.2 ± 0.3	61.5 ± 6.5	7.8 ± 0.2	61.3 ± 5.2	7.4 ± 0.1	85.8 ± 2.7	7.3 ± 0.5	31.7 ± 6.7
WKY-NAC (a)	7.1 ± 0.2	55.3 ± 3.2	7.6 ± 0.2	64.7 ± 4.6	7.6 ± 0.1	84.9 ± 3.3	7.5 ± 0.1	33.5 ± 1.3
WKY-NAC (c)	7.1 ± 0.2	58.5 ± 4.0	7.8 ± 0.3	55.1 ± 7.2	7.6 ± 0.3	75.1 ± 6.2	7.1 ± 0.5	22.5 ± 4.5
SHR	7.4 ± 0.4	44.2 ± 5.4	7.7 ± 0.4	31.2 ± 6.5*	7.1 ± 0.1	89.8 ± 2.5	8.0 ± 1.4	26.6 ± 9.0
SHR-NAC (a)	7.3 ± 0.3	54.5 ± 4.9	7.6 ± 0.3	56.0 ± 7.0†	7.6 ± 0.1	78.4 ± 3.8	7.1 ± 0.4	24.1 ± 5.0
SHR-NAC (c)	7.3 ± 0.3	44.6 ± 5.0	7.8 ± 0.4	58.3 ± 7.8†	7.2 ± 0.1	84.6 ± 3.9	8.0 ± 0.7	23.4 ± 5.9

A23187 = calcium ionophore A23187; Ach = acetylcholine; ISO = isoproterenol; SNP = sodium nitroprusside; a = acute; c = chronic; other abbreviations as in Table 1.

Sensitivity, ED_{50}, is expressed as the negative logarithm of the concentration and the maximal relaxation is expressed as the percentage of the precontractile response to KCl on basal tension.

Values are mean ± SEM $n = 4–8$.

* $P < .01$ SHR *v* WKY; † $P < .05$ NAC *v* untreated.

Statistical analysis: one-way ANOVA in conjunction with Bonferroni correction.

Measurement of IP Formation

Measurement of IP formation was assessed in cultured SMCs as described by us previously.[10] The tritiated IP pool of the aqueous phase represents total IP and was eluted by ion-exchange chromatography (AG1-X8 resin, Bio-Rad Laboratories, Mississauga, Ontario, Canada). The lipid phase was counted to measure the phosphatidylinositol (PIP) lipid pool. The accumulation of IP was expressed as a relative value of (IP/PIP) $\times 10^3$ (arbitrary units) to correct for the variation in the labeling of the lipid pool.

Drugs

All drugs and chemical components of solutions were purchased from Sigma Chemical Company (St. Louis, MO). All drugs were dissolved in water except A23187, which was dissolved in ethanol to give a final concentration of 0.1%. An ethanol concentration (0.1% to 1%) in Krebs-Henseleit solution did not have any effect on 60 mmol/L of KCl-induced vasoconstriction.

Statistical Analysis

The amplitude of the contraction immediately before the addition of different vasodilators was considered to represent 100%. Values are given as mean ± SEM, with *n* indicating the number of observations. Statistical analysis was performed using two-way ANOVA in conjunction with a Bonferroni multiple comparison analysis when *F* values were significant for comparison of three or more groups, and the Student *t* test was used to compare two groups. For multiple comparisons with the same control group, significance was assessed by a one-way ANOVA followed by the Dunnett test. Dose-response curves were fitted to the sigmoidal four-parameter logistic equation by a curve-fitting analysis program (GraphPad Prism for Windows version 2.01; GraphPad Inc., San Diego, CA) to evaluate ED_{50} and maximal relaxation response. This program was also used for all statistical analysis. The ED_{50} values were expressed as the negative logarithm of the drug concentration that produces 50% of the maximal response to each drug. Statistical significance was considered when $P < .05$.

Results

BP and HR

Both MAP and HR were significantly higher in 15-week-old SHR than in WKY (Table 1). Chronic 4-week treatment with NAC produced significant reductions in MAP (−20 mm Hg) and HR (−52 beats/min) only in SHR (Table 1).

NO Pathway

Relaxation to Acetylcholine The mesenteric arterial beds from SHR were significantly less responsive to the endothelium-dependent relaxing effect of ACh than those obtained from WKY ($P < .01$), either with or without NAC treatment, when the total dose-response curves were analyzed with a two-way ANOVA. However, ED_{50} was similar, and the maximal relaxation tended to be lower in SHR compared with WKY but was nonsignificant at $P < .06$ (Table 2). Chronic in vivo or acute in vitro NAC treatments had no significant effects on ACh-induced vasodilation of mesenteric arterial beds in SHR and WKY (Fig. 1, Table 2).

Relaxation to A23187 The responses of mesenteric artery were characterized by a vasorelaxation at low concentrations of A23187 (10^{-10} to 10^{-7} mol/L in SHR and from 10^{-10} to 10^{-6} mol/L in WKY) and a vasoconstriction at higher concentrations (data not shown). As depicted in Fig. 1, the maximal relaxation (note that only the relaxant portion of the curve was considered) of mesenteric artery to A23187 was greater in untreated WKY than in SHR ($P < .01$). Chronic or acute NAC treatment

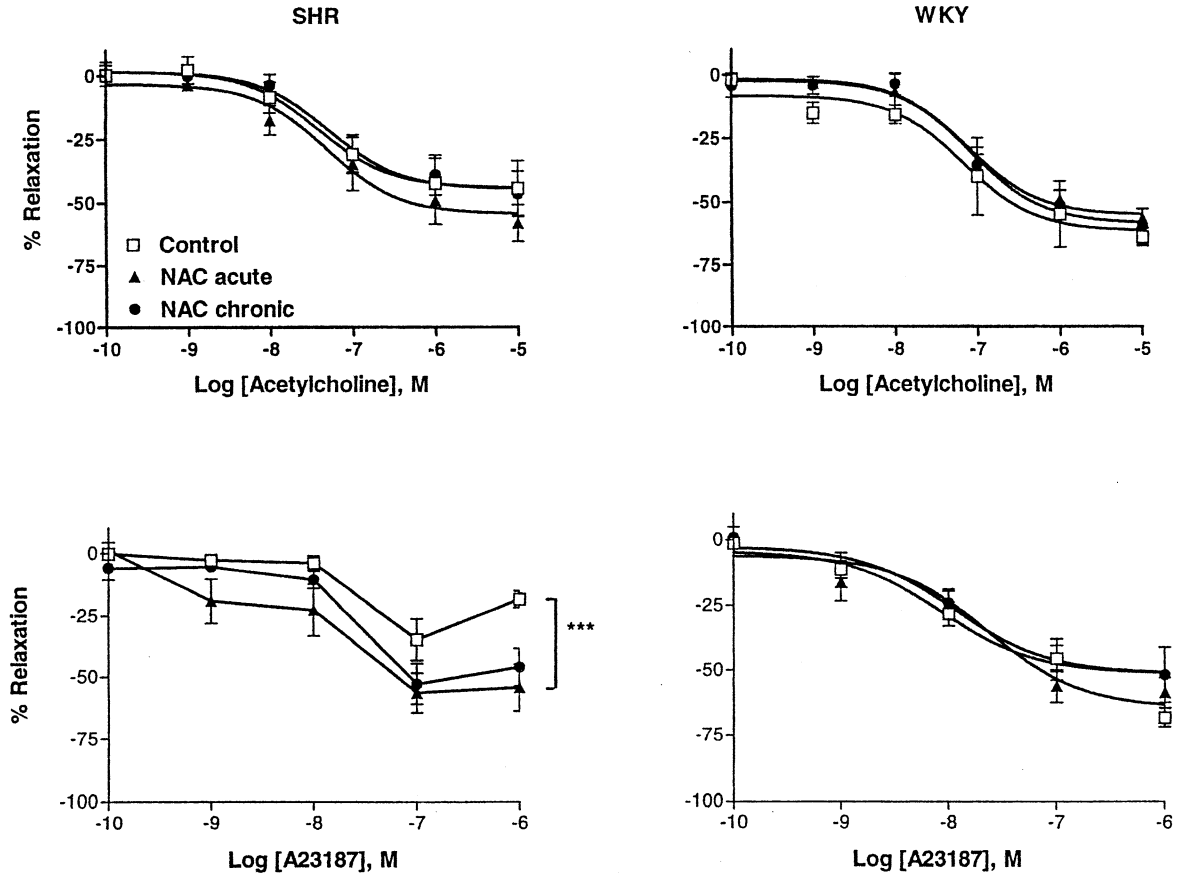

FIG. 1. Dose-response curves to acetylcholine (**upper panels**) and A23187 (**lower panels**) on KCl–induced vasoconstrictions of endothelium intact mesenteric arterial bed before (control) and after acute or chronic 4-week *N*-acetylcysteine (NAC) treatment in spontaneously hypertensive rats (SHR) (**left panels**) and Wistar-Kyoto rats (WKY) (**right panels**). Mesenteric beds were perfused with normal Krebs solution containing KCl at 60 mmol/L. At the plateau of the contraction, the preparation was perfused with Krebs solution containing increasing and cumulative concentrations of acetylcholine or A23187. Data are expressed as means ± SEM of four to eight mesenteric beds and expressed as the percentage of contraction induced by KCl: ***$P < .001$ for chronic or acute NAC treatments v control (two-way ANOVA in conjunction with Bonferroni).

improved the maximal mesenteric bed relaxation of SHR ($P < .05$), whereas the maximal relaxation to A23187 was not modified in vessels from NAC-treated WKY (Table 2).

Relaxation to Sodium Nitroprusside Response curves to SNP were similar in mesenteric artery from untreated SHR and WKY. The NAC treatment did not affect the ED_{50} and the maximal relaxation to SNP in mesenteric artery from SHR and WKY (Table 2).

Effects of NAC on L-NAME–Induced Vasoconstriction in Vivo The increase in MAP after L-NAME injection was lower in SHR compared with WKY ($P < .05$). Chronic NAC treatment did not change the L-NAME–induced increases in MAP in WKY; however, in SHR, it did increase the MAP responses at high doses of L-NAME to the same levels as those observed in WKY ($P < .05$) (Fig. 2).

Adrenergic Functions

Relaxation to Isoproterenol The degree of β-adrenergically induced relaxation by ISO did not differ significantly between WKY and SHR. The dose-response curves to ISO in mesenteric vessels of acutely or chronically NAC treated WKY or SHR rats were similar to those of untreated rats (Table 2).

Constriction to Phenylephrine The vasoconstriction to Phe was greater in SHR than in WKY ($P < .01$) (Fig. 3A). Chronic NAC treatment did not modify the Phe-induced vasoconstriction (Fig. 3A), whereas acute NAC treatment significantly inhibited the Phe-induced vasoconstriction of mesenteric arteries to a greater extent in SHR (66.7% ± 5.4%; $P < .001$) than in WKY (49.2% ± 7.3%; $P < .001$) ($P < .001$ SHR *v* WKY) (Fig. 3B). The inhibition by the acute treatment with NAC was not affected in either group by the presence of L-NAME (Fig. 3B).

Effect of NAC on Free Radical–Induced Contractility and Inositol Phosphate Production

The hypoxanthine-xanthine oxidase (HX-XO), a free radical generating system, induced a significant vasoconstric-

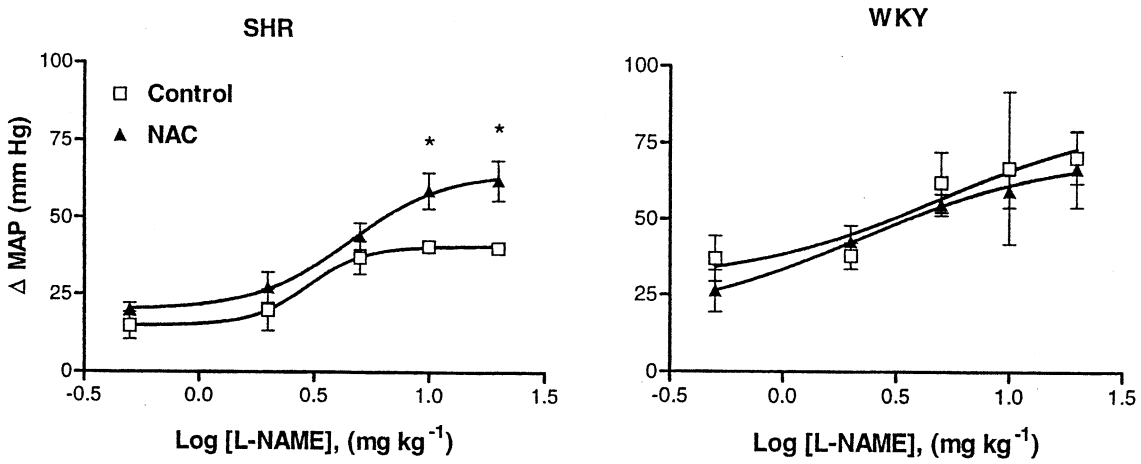

FIG. 2. Dose-response curve of mean arterial pressure (MAP) induced by intravenous administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME) in chronically NAC–treated (4 weeks) and untreated (control) SHR and WKY rats. Other abbreviations as in Fig. 1. Data are expressed as mean ± SEM of four to six mesenteric beds. * $P < .05$ for NAC *v* control (two-way ANOVA in conjunction with Bonferroni).

tion of mesenteric vessels (Fig. 4A and 4B) and in the IP production of cultured vascular SMC. The acute NAC treatment significantly inhibited the HX-XO–induced IP formation in a concentration-dependent manner (Fig. 4C) and significantly inhibited HX-XO–induced vasoconstriction in mesenteric beds with or without endothelium ($P < .001$) (Fig. 4A and 4B).

Discussion

The findings of the present study can be summarized as follows: 1) chronic NAC treatment decreased MAP and HR in SHR; 2) chronic or acute treatments with the antioxidant NAC enhanced the responses of mesenteric beds from SHR to the calcium ionophore A23187, an endothelium-dependent vasodilator that stimulates NO release; 3) acute treatment with NAC produced NO-independent impairment of α-adrenergically induced vasoconstriction that was stronger in mesenteric beds of SHR than those of WKY; 4) acute NAC treatment counteracted the IP-induced formation and constrictive effect of free radicals production induced by the HX-XO reaction.

Effects of NAC on NO Pathway

In the present study, the endothelium-dependent vasodilation and the activity of the NOS pathway as evaluated by ACh and the calcium ionophore A23187, a potent releaser of NO, were found to be impaired in mesenteric beds from SHR compared with WKY. Considering that altered vasodilatory functions in SHR could be the result of enhanced oxidative stress, it was expected that NAC treatment would improve endothelial dependent vasodilation. However, improvement in vasodilation was observed only with A23187 but not with ACh. The discrepancy between the effect of NAC on ACh- and A23187-induced vasodilations could be explained by the fact that ACh stimulates NO production only from endothelial NOS (eNOS), whereas A23187 stimulates NO release from both neuronal NOS (nNOS) and eNOS. Indeed, nNOS is more susceptible than eNOS to produce superoxide anion and peroxinitrite,[14] which can be scavenged efficiently by antioxidants such as NAC.

In other studies in which NAC treatment was found to improve endothelium-dependent relaxation to ACh in isolated mesenteric arteries[5] and aorta,[6] precontractions were induced either with phenylephrine or U-46619, a thromboxane A_2 mimetic. In the present study, an effect on endothelium-derived hyperpolarizing factor (EDHF) pathway may be excluded, as the relaxant effects were measured in the presence of K^+-induced concentrations that decrease EDHF effects. Therefore, the improved ACh vasorelaxation observed in those studies could be attributed to an improved EDHF effect rather than to an improvement in the NO pathway.

Moreover, the enhanced A23187-induced vasodilation by NAC treatment and the absence of an effect of that treatment on SNP-induced vasodilation in isolated vessels suggest that the effect of NAC is mediated either by an activation of NOS or by an inhibition of NO degradation. To verify the contribution of NOS pathway in the MAP reduction by NAC, the pressor effect of intravenous injections of a NOS inhibitor, L-NAME, were evaluated. The increases in MAP after L-NAME injection were lower in untreated SHR compared with WKY, suggesting impaired NOS pathway activity in SHR. Because increases in MAP, at high L-NAME concentrations, were enhanced in NAC-treated SHR and became similar to increases observed in WKY, these results suggest the restoration of the sensitivity of the NOS pathway in SHR by chronic treatment with NAC.

The improvement by NAC treatment of the activity of NOS pathway as evaluated by the response to the calcium ionophore A23187 or L-NAME could be mediated

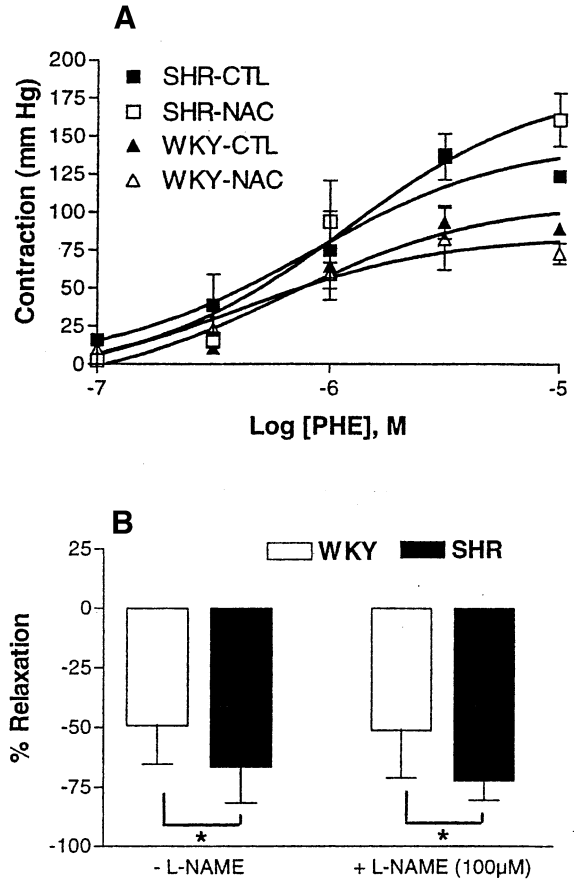

FIG. 3. A) Dose-response curves to phenylephrine (PHE) of endothelium intact mesenteric arterial bed before (control) and after a chronic NAC treatment in SHR and WKY rats. The preparation was perfused with a Krebs solution containing increasing and cumulative concentrations of PHE. **B**) Effects of acute treatment with NAC on PHE-induced contraction of mesenteric arterial bed in SHR and WKY rats. The acute effect of NAC was assessed in mesenteric beds in the absence (−L-NAME) or the presence (+L-NAME, 100 μmol/L). Data are expressed as mean ± SEM of four to eight mesenteric beds: * $P < .05$ SHR *v* WKY (Student *t* test). Other abbreviations as in Figs. 1 and 2.

through an antioxidant effect. Indeed, in preliminary experiments, the superoxide scavenging property of NAC was clearly demonstrated in cultured SMCs from SHR and WKY. Moreover, NAC is the precursor of another antioxidant compound, glutathione.[15] It has also been demonstrated that chronic NAC treatment increased the activities of glutathione (GSH) peroxidase and glutathione disulfide (GSSG) reductase and decreased the aortic GSSG/GSH balance in SHR.[6,16] Furthermore, it has been demonstrated in genetically prehypertensive or hypertensive rats, in DOCA-salt–treated rats, and in rats made hypertensive by aortic banding, that higher basal or NADH-stimulated production of superoxide anion is present in the aorta or coronary arteries of those animals.[17–20] These results could thus explain the specific preferential and more potent effects of NAC that were observed in mesenteric arterial beds from SHR rather than in those from WKY.

Effects of NAC on Adrenergic Pathways

The dose-response curves of the β-receptor agonist ISO in mesenteric beds were similar in mesenteric artery from SHR compared with WKY, and were not modified by chronic or acute NAC treatments. The absence of effect of the treatment with NAC on β-adrenergic pathways in vascular bed from SHR suggests that this pathway does not seem to be altered by free radicals produced in mesenteric arteries.

On the other hand, the Phe-induced vasoconstriction that was found to be greater in mesenteric arteries of SHR compared with WKY was acutely inhibited by NAC in SHR and WKY. The inhibition was stronger in SHR compared with WKY. Furthermore, our results obtained from mesenteric beds in the absence and presence of L-NAME showed that this effect of NAC on α_1-induced vasoconstriction was independent of the NOS pathway.

Effects of NAC on Free Radical–Induced IP Production

Because it has been demonstrated in isolated smooth muscle cells that superoxide anions induce an important IP production that is greater in SHR,[10] it may be postulated that the effect of NAC on Phe-induced vasoconstriction may be due to an inhibition of free radical production and to an associated reduction in superoxide-induced IP production. The increased contractility and IP production induced by the free radical generating system HX-XO was completely inhibited by NAC (10 mmol/L) in the presence or absence of endothelium. These results strongly suggest that NAC could inhibit the HX-XO–induced contractility and IP production by scavenging free radicals. The greater production of free radicals in vessels from SHR than in those from WKY could explain the stronger effect observed with NAC on Phe-induced vasoconstriction in arterial beds from SHR.[15]

Effects of NAC on BP and HR

This study reports that a chronic, 4-week NAC treatment attenuated hypertension and decreased HR in SHR. These findings regarding BP are in agreement with earlier experimental and clinical studies that have previously reported the hypotensive effects of NAC[7,21] and its vasorelaxant properties,[5,6] The present results suggest that the hypotensive effect of NAC is most likely due to the potentiation of the NO pathway. These findings are in accordance with those of Fenoy et al,[22] who demonstrated an antihypertensive effect of NAC in one-kidney, one-clip induced hypertension through a NO-dependent mechanism. It has also been shown that NAC can potentiate the antihypertensive response to angiotensin converting enzyme inhibitors in SHR and in essential hypertension by a NO-dependent mechanism.[8] Moreover, it has been shown that NAC enhances the hypotensive effect of ACh in vivo in normotensives rats and in SHR through a NO-dependent mechanism.[7] In the present study, the absence of effect of

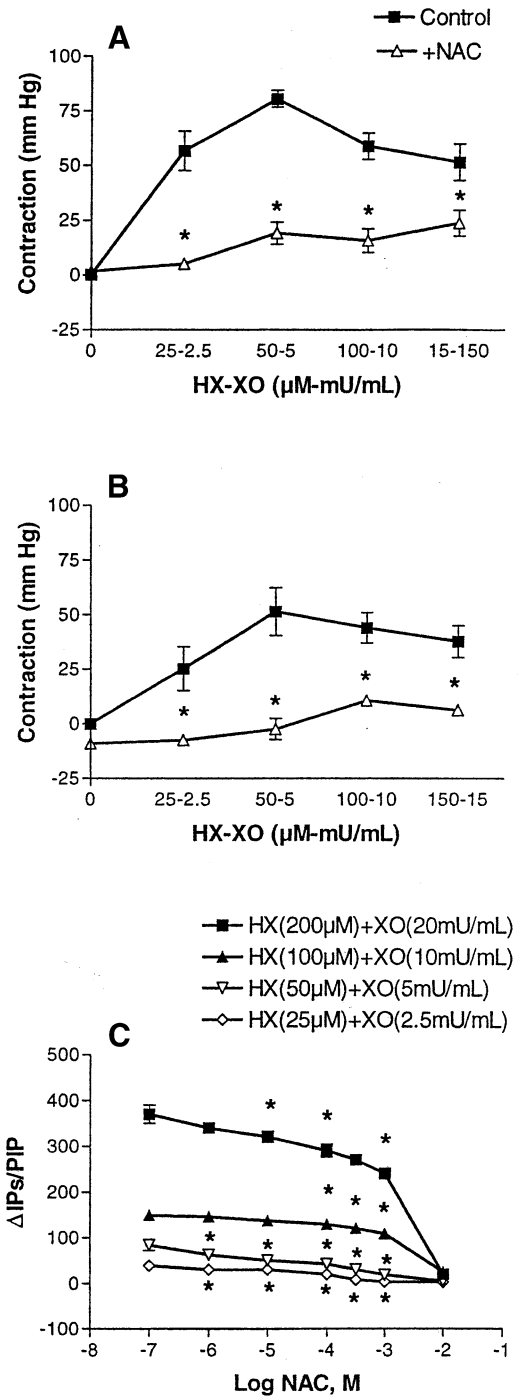

FIG. 4. The effect of NAC on the contraction of mesenteric beds with (**A**) or without (**B**) endothelium, independently of the hypertensive state by hypoxanthine-xanthine oxidase (HX-XO)–induced formation of free radicals in Sprague-Dawley rats. Mesenteric beds were perfused with normal Krebs solution containing KCl at 60 mmol/L. At the plateau of the contraction, the preparation was perfused with a Krebs solution containing NAC (10 mmol/L) for 20 min followed by increasing and cumulative concentrations of HX-XO. Data points represent means of pressure over KCl-induced contraction ± SEM ($n = 8$). *$P < .05$ (paired t-test). **C**) Effect of NAC on the inositol phosphate (IP) formation by HX-XO–induced free radicals production in cultured smooth muscle cells from Sprague-Dawley rats. After pretreatment with NAC at different concentrations for 20 min, HX-XO–induced IP formation was progressively and dose-dependently inhibited in smooth muscle cells after superoxide production with xanthine-xanthine oxidase. Δ indicates absolute change in (IP/PIP) × 10^3 after substraction of basal values. Data points represent means ± SEM. *$P < .05$ (one-way ANOVA in conjunction with Dunnett test), compared with initial IP production by various concentrations of HX-XO. $n = 4$ to 8. Other abbreviation as in Figs. 1–3.

chronic NAC treatment on β-adrenergic vascular functions suggest that such mechanism is not involved in the hypotensive effect of the chronic NAC treatment. However, the involvement of the α_1-adrenergic vascular component in the hypotensive effect of NAC needs to be clarified. The decrease in HR induced by a chronic NAC treatment may be due to a decrease in sympathetic tone.[21]

In conclusion, acute and chronic treatments with NAC were found to improve the potency of the NOS pathway, an effect that is most likely mediated by its antioxidant properties. Acute treatment with NAC was also found to inhibit the α_1-adrenergic–induced vasoconstriction in mesenteric beds independently of its effects on the NOS pathway. Therefore, the hypotensive effect of NAC in SHR could be explained by the potentiating effect of NAC on vasodilatory mechanism in that experimental model of hypertension, as well as its possible inhibitory effect on adrenergic vasoconstrictory mechanism, which remains to be elucidated in vivo.

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