

Effect of acute administration of vitamin C on muscle sympathetic activity, cardiac sympathovagal balance, and baroreflex sensitivity in hypertensive patients^{1–3}

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ABSTRACT

Background: Essential hypertension is characterized by both increased oxidative stress and sympathetic traffic. Experimental studies have shown that reactive oxygen species can modulate autonomic activity.

Objective: The aim of this study was to determine whether acute administration of the antioxidant vitamin C modifies sympathetic nerve activity in essential hypertension.

Design: Thirty-two untreated patients with essential hypertension and 20 normotensive subjects received vitamin C (3 g intravenously in 5 min) or vehicle. Heart rate, noninvasive beat-to-beat blood pressure, and muscle sympathetic nerve activity (microneurography) were monitored at baseline and up to 20 min after the infusion. Spectral analysis of RR interval variability and spontaneous baroreflex sensitivity were also computed.

Results: Vitamin C infusion significantly lowered blood pressure in hypertensive patients but not in normotensive subjects (maximal changes in systolic blood pressure: -4.9 ± 10.1 compared with -0.7 ± 4.0 mm Hg, respectively; $P < 0.05$). Moreover, muscle sympathetic nerve activity was significantly reduced after vitamin C infusion in hypertensive patients (from 53.3 ± 12.2 to 47.4 ± 11.5 bursts/100 heart beats; $P < 0.01$) but not in healthy subjects (from 42.0 ± 10.1 to 42.7 ± 11.8 bursts/100 heart beats; NS). On the contrary, in 16 hypertensive patients, sodium nitroprusside in equi-depressor doses induced a significant increase in muscle sympathetic nerve activity compared with vitamin C ($+10.0 \pm 6.9$ bursts/100 heart beats). Sympathovagal balance and spontaneous baroreflex sensitivity were restored during vitamin C infusion in hypertensive subjects.

Conclusions: These results indicate that acute administration of vitamin C is able to reduce cardiovascular adrenergic drive in hypertensive patients, which suggests that oxidative stress is involved in the regulation of sympathetic activity in essential hypertension. *Am J Clin Nutr* 2012;96:302–8.

INTRODUCTION

In essential hypertension, increased sympathetic nervous system (SNS)⁴ activity is one of the main mechanisms responsible for the pathogenesis of the disease and the development of target organ damage (1, 2). However, the mechanisms underlying such alterations are not yet fully understood. Another pathophysiologic characteristic of essential hypertension is the presence of increased oxidative stress, reduced activity of antioxidant enzymes, and decreased plasma concentrations of antioxidant vitamins (3). The altered redox status seems to play a role in the

pathogenesis of hypertension and in the development of atherosclerotic damage (3).

Interestingly, oxidative stress appears to be involved in the enhanced central sympathetic outflow present in different experimental models of hypertension: intravenous or intracerebral infusion of antioxidants is able to reduce blood pressure (BP) and adrenergic overdrive in hypertensive animals, with little or no effect in control animals (4–7). Few data are available on the interaction between oxidative stress and SNS in humans. In healthy subjects, vitamin C infusion exerts no effect on muscle sympathetic nervous activity (MSNA), evaluated by microneurography (8). On the other hand, in heart failure—a disease characterized by increased sympathetic activity—antioxidants are effective in restoring vagal control of heart rate and improving baroreflex sensitivity acutely but not chronically (9, 10). Thus, the possibility exists that oxidative stress contributes to sympathetic activation characterizing essential hypertension. Accordingly, the aim of the current study was to evaluate the effect of acute systemic administration of the reactive oxygen species scavenger vitamin C on SNS activity directed to the muscle vasculature and on sympathovagal regulation of heart rate fluctuations in patients with essential hypertension.

SUBJECTS AND METHODS

Population

The current study included 48 never-treated patients with essential hypertension, who were recruited in our outpatient

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⁴ Abbreviations used: BP, blood pressure; FRAP: ferric-reducing antioxidant power; HF, spectral power of the high-frequency band; LF, spectral power of the low-frequency band; MSNA, muscle sympathetic nervous activity; SNS, sympathetic nervous system.

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Hypertension Unit starting in October 2007. Inclusion criteria were office systolic BP values ≥ 140 mm Hg and/or office diastolic BP values ≥ 90 mm Hg, which were confirmed on repeated occasions within 1 mo according to current guidelines. Exclusion criteria were as follows: secondary hypertension, history of cardiovascular disease, diabetes mellitus, alcohol consumption > 50 g/d, current smoking, current use of mineral or vitamin supplements, and major noncardiovascular comorbidities. Patients never received any antihypertensive treatment and did not take any medication known to interfere with cardiovascular variables in the 2 wk before the experimental session. Twenty age- and sex-matched normotensive, healthy volunteers were also included as a control group. The study protocol was approved by the local ethical committee of our institution and was in accordance with guidelines in the Declaration of Helsinki. Patients gave their written informed consent to participation in the study after an explanation of its nature and purpose.

Measurements

In each subject, a detailed medical history and physical examination were carried out; office BP (3 measurements in the sitting position by means of the automatic device Omron 705IT), heart rate, anthropometric variables, and routine blood sample assays were obtained before inclusion in the study.

Hemodynamic variables

Noninvasive beat-to-beat BP was obtained through a finger photoplethysmographic device (Portapres; Finapres Medical System), which provides accurate and reproducible systolic and diastolic BP values and allows the evaluation of acute variations in total peripheral resistances (11). Heart rate was obtained by means of one transthoracic electrocardiogram lead (Biotach; Gould Electronics).

MSNA

Microneurography was used to obtain multiunit recording of efferent postganglionic MSNA. Briefly, a tungsten microelectrode with an uninsulated 1–5- μ m-diameter tip (Medical Instruments, University of Iowa) was transcutaneously inserted in the right or left peroneal nerve posterior to the fibular head, as previously described (12). The signal was integrated with a 0.1-s time constant, amplified with a gain of 50,000–80,000, band-pass filtered (700–2000 Hz), and acquired at 1000 Hz through a digital acquisition system (ACQ-16; Gould Electronics). MSNA was identified according to criteria outlined in previous studies (12, 13). Neurograms thereby obtained were recorded together with BP and heart rate by means of dedicated computer software (Ponemah; LDS). MSNA was quantified as bursts/min and bursts/100 heart beats. MSNA was analyzed by visual inspection by a single investigator blinded to the drug infused. During the recording, respiration rate was monitored through a strain gauge pneumograph (Pneumotrace; Gould Electronics), positioned at the midchest level, to exclude from the data analysis any time interval in which respiratory rhythm alterations were present.

Spectral analysis of RR interval and spontaneous baroreceptor sensitivity

The electrocardiogram was acquired and digitalized together with neural and BP recordings at 1000 Hz, as described above. The RR-interval time series was obtained with a QRS complex detection algorithm based on threshold on derivative signal by selecting the normal-to-normal RR intervals through a predictive filter, as previously described (14). Spectral analysis of the RR-interval time series was obtained according to the Welch method by using a frequency resolution of 0.0167 Hz; window length, overlapping, and the number of intervals were automatically adjusted according to the effective time duration of the 300-s epoch. The following parameters in the frequency domain were considered: spectral power in the low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.4 Hz) bands, both expressed in absolute values and in normalized units—the LF/HF ratio (15).

The sensitivity of the baroreceptor–heart rate reflex was estimated by the frequency-domain analysis of spontaneous variability of systolic BP and RR interval. In particular, the square-root ratio of RR and systolic BP powers (α -index) in both the HF and LF bands were used to estimate baroreflex gain. The α -index was calculated after verification of a high degree of coherence (> 0.5).

Plasma assays

Plasma norepinephrine and adrenaline were assayed by HPLC. Malondialdehyde, a marker of lipid peroxidation, was assayed by spectrophotometric assay (Bioxitech LPO-586; OXIS International Inc). Ferric-reducing antioxidant power (FRAP), an index of total plasma antioxidant capacity, was assayed by spectrophotometric assay.

Experimental protocol

Study 1: effect of vitamin C on SNS activity

All experimental sessions were performed in the morning, and subjects were asked to avoid drinking caffeine- and alcohol-containing beverages and eating for the 12 h before the study. The subjects were studied in the supine position in a quiet and comfortable room. A polyethylene cannula was inserted in a forearm vein for blood samples and drug administration, and the microelectrodes for MSNA recording and the other measuring devices were positioned. After a 30-min interval, BP, heart rate, respiration rate, and MSNA were continuously monitored and recorded. Thirty-two hypertensive patients and 20 healthy, normotensive subjects were randomly assigned to infusion of the antioxidant vitamin C (Roche), at a dose of 3 g (15 mL) in 5 min, or vehicle (0.9% saline, 15 mL in 5 min). This dose was chosen because it is able to induce fast and consistent increases of plasma vitamin C concentrations, as previously reported (10, 16, 17). All parameters were continuously recorded for a 5-min baseline period, during the 5-min period of infusion, and for 15 min thereafter. Venous blood samples were collected at baseline and at the end of the recording.

Study 2: effect of sodium nitroprusside infusion in hypertensive patients

To exclude the possibility that changes in BP induced by vitamin C were merely responsible for baroreflex-mediated changes



in sympathetic nerve activity, another vasodilator acting with a different mechanism, sodium nitroprusside, was used in 16 additional hypertensive patients matched for clinical characteristics with the hypertensive group of study 1. In an experimental session superimposable to those described above, after a 30-min interval, sodium nitroprusside (Malesci; Firenze) was infused intravenously for 20 min at doses capable of inducing the same BP reduction obtained with vitamin C ($0.4\text{--}0.8 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Data analysis

All statistical analyses were performed by using NCSS 2004. Variables were expressed as means \pm SDs if normally distributed or medians (interquartile ranges) otherwise. Differences between normotensive and hypertensive subjects were analyzed by using an unpaired Student's *t* test, with log transformation for non-normally distributed variables; categorical variables were analyzed by chi-square test. Systolic and diastolic BP, total peripheral resistances, and MSNA were obtained in every patient and averaged for intervals of 5 min; RR interval spectral analysis and BRS parameters were calculated for 5-min time intervals as well and log transformed for analysis when appropriate. Differences in means in comparison with baseline and between drug and placebo infusions were calculated by ANOVA for repeated measures, considering time intervals and drug type as factors, and Bonferroni post hoc multiple comparison test by using log transformation when appropriate. A *P* value <0.05 was used to define statistical significance. The sample size was calculated on preliminary data to reject the hypothesis of no difference between the vitamin C and placebo effect on MSNA in patients with essential hypertension with a power of 0.8 and a type I error probability of 0.05.

RESULTS

Baseline values

The clinical characteristics of patients with essential hypertension and normotensive, healthy volunteers are shown in **Table 1**. As expected, BP values and MSNA were higher in patients

with essential hypertension than in normotensive, healthy volunteers, whereas normotensive, healthy volunteers and patients with essential hypertension were not significantly different for age, sex, heart rate, BMI, plasma glucose, lipid profile, and renal function. The clinical characteristics of hypertensive patients enrolled in studies 1 and 2 did not differ significantly (**Table 1**).

Hemodynamic variables

In patients with essential hypertension, systolic and diastolic BP were significantly reduced by vitamin C infusion both during and after the infusion, whereas placebo did not exert any pressor effect (**Figure 1**, A and C). In contrast, vitamin C did not modify BP in normotensive, healthy volunteers, as shown in **Figure 1**, B and D. The maximal absolute changes (Δ) in BP values were greater in patients with essential hypertension than in normotensive, healthy volunteers (Δ : -4.9 ± 10.1 compared with -0.7 ± 4.0 mm Hg for systolic BP; Δ : -5.6 ± 8.3 compared with -0.8 ± 4.9 mm Hg for diastolic BP; *P* < 0.05 compared with normotensive, healthy volunteers for all). At baseline total peripheral resistances were higher in patients with essential hypertension than in normotensive, healthy volunteers (1.13 ± 0.66 compared with 0.88 ± 0.52 MU; *P* = 0.002). During vitamin C infusion, total peripheral resistances were not modified in either group, but in the postinfusion period a decrease was shown only in patients with essential hypertension (from 1.17 ± 0.68 to 1.01 ± 0.63 MU; *P* < 0.05).

According to the protocol of study 2, sodium nitroprusside infusion in a group of hypertensive patients caused a BP lowering effect similar to that of vitamin C, for both systolic and diastolic BP values (Δ : -6.2 ± 3.1 mm Hg for systolic BP; Δ : -4.7 ± 6.6 mm Hg for diastolic BP; *P* < 0.05 compared with vitamin C for both; **Figure 1**, A and C).

Sympathetic nerve activity

In patients with essential hypertension, MSNA remained unchanged during vitamin C infusion then started to decrease,

TABLE 1
Clinical characteristics of the study 1 and 2 populations¹

	Study 1		Study 2
	Normotensive subjects (<i>n</i> = 16 M, 4 F)	Hypertensive patients (<i>n</i> = 22 M, 10 F)	Hypertensive patients (<i>n</i> = 12 M, 4 F)
Age (y)	45.7 \pm 7.0	47.1 \pm 8.0	47.4 \pm 6.2
Systolic BP (mm Hg)	123.5 \pm 9.1	144.8 \pm 13.4*	145.4 \pm 8.1*
Diastolic BP (mm Hg)	76.5 \pm 9.5	91.9 \pm 10.5*	91.1 \pm 8.6*
MSNA (bursts/100 heart beats)	43.8 \pm 8.2	54.3 \pm 12.2*	52.9 \pm 10.7*
MSNA (bursts/min)	28.4 \pm 4.4	34.9 \pm 7.2	36.3 \pm 8.6
Heart rate (beats/min)	65.3 \pm 10.3	66.2 \pm 9.5	70.6 \pm 12.7
BMI (kg/m ²)	27.0 \pm 3.6	26.4 \pm 3.3	25.5 \pm 2.7
Total cholesterol (mmol/L)	5.5 \pm 1.0	5.6 \pm 1.2	5.2 \pm 0.9
HDL cholesterol (mmol/L)	1.3 \pm 0.4	1.4 \pm 0.3	1.4 \pm 0.4
LDL cholesterol (mmol/L)	3.2 \pm 1.3	3.2 \pm 0.9	3.2 \pm 0.8
Triglycerides (mmol/L)	1.4 \pm 0.5	1.4 \pm 0.6	1.2 \pm 0.4
Blood glucose (mmol/L)	5.2 \pm 0.4	5.1 \pm 0.6	4.9 \pm 0.5
Serum creatinine (μ mol/L)	80.0 \pm 12.2	82.8 \pm 18.0	84.0 \pm 17.7

¹ All values are means \pm SDs. *Significantly different from normotensive subjects, *P* < 0.05 (unpaired Student's *t* test; chi-square test for categorical variables). BP, blood pressure; MSNA, muscle sympathetic nervous activity.

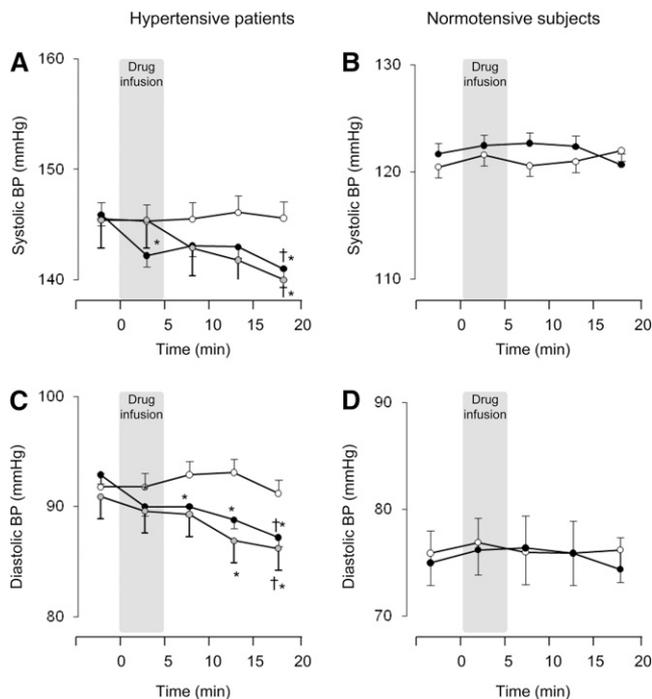


FIGURE 1. Mean (\pm SEM) systolic (A and B) and diastolic (C and D) BPs during the experimental sessions in hypertensive and normotensive subjects in the placebo arm (\circ), the vitamin C arm (\bullet), and the sodium nitroprusside arm (gray circles). ANOVA for repeated measures, considering time intervals and drug type as factors with a Bonferroni post hoc multiple comparison test: *Significantly different from baseline, $P < 0.05$; †Significantly different from placebo, $P < 0.05$. Systolic BP: $P = 0.046$ for the time-drug interaction in hypertensive patients and 0.75 in normotensive subjects. Diastolic BP: $P = 0.003$ for the time-drug interaction in hypertensive patients and 0.56 in normotensive subjects. BP, blood pressure.

reaching the minimum value after 20 min (from 53.3 ± 12.2 to 47.4 ± 11.5 bursts/100 heart beats; $P < 0.01$), whereas in normotensive, healthy volunteers, MSNA was unmodified (from 42.0 ± 10.1 to 42.7 ± 11.8 bursts/100 heart beats) (Figure 2, A and B). The results were similar when MSNA was expressed in bursts per minute (patients with essential hypertension: from 34.6 ± 7.9 to 30.5 ± 7.6 bursts/min, $P < 0.01$; normotensive, healthy volunteers: from 27.1 ± 4.6 to 26.9 ± 5.7 bursts/min; NS). The maximal absolute decrease in MSNA was greater in patients with essential hypertension than in normotensive, healthy volunteers: -5.8 ± 3.9 compared with $+1.1 \pm 8.0$ bursts/100 heart beats ($P < 0.01$) and -4.2 ± 2.5 compared with -0.2 ± 1.9 bursts/min ($P < 0.01$). MSNA was not altered by placebo infusion in patients with essential hypertension or in normotensive, healthy volunteers (Figure 2, A and B).

At variance with vitamin C, MSNA was significantly increased by sodium nitroprusside infusion ($+10.0 \pm 6.9$ bursts/100 heart beats; $P < 0.05$ compared with vitamin C), because of the baroreflex-mediated response to BP lowering (Figure 2A).

RR interval spectral analysis and spontaneous baroreflex sensitivity

Complete results concerning RR interval spectral analysis and spontaneous baroreflex sensitivity in study 1 were reported elsewhere (see online supplemental material under "Supplemental data" in the online issue). At baseline, the RR interval

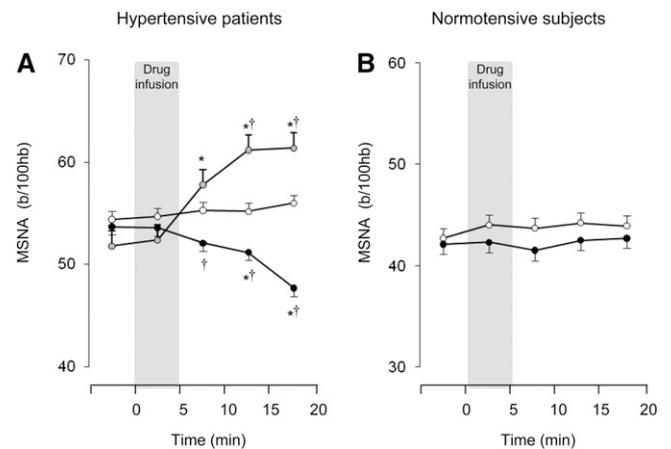


FIGURE 2. Mean (\pm SEM) MSNA in hypertensive patients (A) and in normotensive subjects (B) in the placebo arm (\circ), the vitamin C arm (\bullet), and the sodium nitroprusside arm (gray circles). ANOVA for repeated measures, considering time intervals and drug type as factors, with a Bonferroni post hoc multiple comparison test: *Significantly different from baseline, $P < 0.05$; †Significantly different from placebo, $P < 0.05$. $P = 0.003$ for the time-drug interaction in hypertensive patients and 0.73 in normotensive subjects. b/100 hb, bursts per 100 heart beats; MSNA, muscle sympathetic nervous activity.

tended to be shorter in patients with essential hypertension than in normotensive, healthy volunteers ($P = 0.09$). Moreover, hypertensive patients showed a reduced HF power, when expressed as absolute values ($P < 0.01$) or as normalized units ($P < 0.001$), and an increased normalized LF power and LF/HF ratio ($P < 0.001$ for both).

In patients with essential hypertension, the heart rate decreased and the RR interval increased during vitamin C infusion in comparison with placebo; vitamin C also showed a shift in the sympathovagal balance, namely a reduction of the LF component of RR interval variability and of the LF/HF ratio [from 3.37 (1.37–5.93) to 1.95 (1.42–4.62); $P = 0.02$]. None of these effects were encountered when the same protocol was applied to healthy subjects.

At baseline, patients with essential hypertension tended to have a reduced spontaneous baroreflex sensitivity compared with normotensive, healthy volunteers (α -index LF, $P = 0.08$; α -index HF, $P < 0.05$). Baroreflex sensitivity increased during vitamin C infusion in patients with essential hypertension (α -index LF: from 7.1 ± 3.2 to 8.7 ± 3.2 ms/mm Hg, $P = 0.03$; α -index HF: from 7.0 ± 3.4 to 9.7 ± 4.9 ms/mm Hg, $P = 0.04$) but not in normotensive, healthy volunteers or during placebo infusion (for complete data, see online supplemental material under "Supplemental data" in the online issue).

During sodium nitroprusside infusion, the heart rate increased (from 70.6 ± 12.7 to 84.5 ± 15.9 beats/min) and the RR interval decreased (from 867 ± 168 to 741 ± 188 ms), $P < 0.05$ for both. The LF/HF ratio increased from 3.6 (1.2–5.4) to 5.1 (2.7–7.6), whereas baroreflex sensitivity decreased (α -index LF: from 6.8 ± 2.8 to 5.8 ± 2.5 ms/mm Hg; α -index HF: from 6.9 ± 2.9 to 5.6 ± 2.3 ms/mm Hg; $P = 0.09$ and $P < 0.05$ compared with baseline, respectively).

Neurohumoral and oxidative stress variables

Humoral variables from study 1 are shown in Table 2. Patients with essential hypertension had greater baseline malondialdehyde

concentrations than did normotensive, healthy volunteers but similar FRAP concentrations. Infusion of vitamin C was effective at reducing malondialdehyde only in patients with essential hypertension, whereas the reduction was not significant in normotensive, healthy volunteers ($P = 0.17$). FRAP increased significantly by vitamin C both in patients with essential hypertension and in normotensive, healthy volunteers. At baseline, plasma norepinephrine was higher in patients with essential hypertension than in normotensive, healthy volunteers, whereas plasma adrenaline was similar between these groups. Vitamin C infusion did not significantly modify plasma norepinephrine or adrenaline in normotensive, healthy volunteers or in patients with essential hypertension.

Plasma norepinephrine and adrenaline were not modified by sodium nitroprusside infusion (from 1.9 ± 0.6 to 2.0 ± 0.5 nmol/L and from 138 ± 81 to 157 ± 113 pmol/L, respectively; NS) nor were oxidative stress variables (malondialdehyde: from 4.1 ± 1.8 to 4.2 ± 1.8 $\mu\text{mol/L}$; FRAP: from 780 ± 200 to 924 ± 400 $\mu\text{mol/L}$; NS for both).

DISCUSSION

The main finding of the current study was that acute administration of the antioxidant vitamin C was able to blunt sympathetic activation in hypertensive patients, which suggests that oxidative stress plays a significant role in the control of sympathetic outflow in essential hypertension.

Effect of vitamin C on muscle sympathetic activity and cardiac sympathovagal balance

In the current experimental conditions, we used different techniques to assess autonomic function at different levels: microneurography, the only available technique able to directly record efferent postganglionic sympathetic activity in humans; RR interval variability, giving information on sympathovagal modulation of sinoatrial node activity; and plasma catecholamines. Intravenous, high-dose administration of vitamin C corrected, at least in part, the well-established (1, 2, 18) derangement in autonomic cardiovascular regulation highlighted by the above-mentioned techniques in hypertensive patients, whereas it was ineffective in normotensive individuals. These results agree with previous evidence obtained in both animals (4–7) and humans (8). In particular, Bell et al (8) found no hemodynamic or sympathetic effect of vitamin C infusion in young or aged healthy

subjects. Thus, oxidative stress, which does not seem to contribute either to basal sympathetic tone in physiologic conditions or to autonomic alterations accompanying aging, is specifically involved in autonomic dysregulation in the setting of hypertension. This hypothesis is supported by experimental studies that showed increased oxidative stress in neurons of rostral-ventrolateral medulla in hypertensive but not in normotensive rats (19, 20). Moreover, the acute correction of oxidative stress, with vitamin C (7) or different antioxidants (4, 5, 21), infused both intravenously and intracerebrally, can reduce SNS activity. Selective vitamin C injection in the rostral-ventrolateral medulla in 2 kidney–1 clip rats reproduces all hemodynamic and sympathoinhibitory effects of its systemic administration (7). Vitamin C can cross the blood-brain barrier, even if in small quantities, as ascorbic acid and in the oxidized form dehydroascorbate (22). Furthermore, the interaction between SNS and oxidative stress may occur not only in the brainstem but also in the peripheral nervous system, such as preganglionic neurons (23), sympathetic ganglia (24), and peripheral nerves (25). It should be also noted that plasma norepinephrine behavior was unmodified after vitamin C and sodium nitroprusside infusions. Previous studies showed a dose-dependent, acute increase in plasma norepinephrine after sodium nitroprusside infusion at higher doses than those used in this study (26). Thus, it is conceivable that the smaller adrenergic activation obtained in our study was not detected by norepinephrine assay. Discrepancies can be also explained by the lower sensitivity and reproducibility of this method for assessing SNS activity (27).

Effect of vitamin C on baroreflex sensitivity

At baseline, essential hypertensive patients showed reduced spontaneous baroreflex sensitivity, as previously shown (28). This alteration is thought to be caused by a loss of carotid elastic properties, by altered mechanotransduction at the baroreceptor level, or by central neural mechanisms (29). The observed increase in both the LF and HF α -indexes during vitamin C administration indicates an improved sensitivity of sinoatrial node to spontaneous BP oscillations. This finding may reflect the ability of this antioxidant molecule to restore an underlying dysfunction within the baroreflex pathway, as previously shown in healthy older men (30). The site of action has not been univocally identified yet, although a direct effect of antioxidant compounds was shown in isolated carotid sinuses from

TABLE 2

Neurohormonal and oxidative stress variables in study 1¹

	Hypertensive patients				Normotensive subjects			
	Baseline	Vitamin C	Placebo	<i>P</i> value ²	Baseline	Vitamin C	Placebo	<i>P</i> value ²
Norepinephrine (nmol/L)	$2.0 \pm 0.7^{\dagger}$	1.9 ± 0.7	2.1 ± 1.2	0.80	1.6 ± 0.7	1.7 ± 0.6	1.4 ± 0.5	0.27
Adrenaline (pmol/L)	121 ± 63	158 ± 147	184 ± 136	0.26	162 ± 93	141 ± 63	162 ± 120	0.17
Malondialdehyde ($\mu\text{mol/L}$)	$3.7 \pm 2.1^{\dagger}$	$2.8 \pm 1.3^*$	3.5 ± 1.8	0.04	2.6 ± 1.3	2.0 ± 0.7	2.4 ± 2.1	0.17
FRAP ³ ($\mu\text{mol/L}$)	726 ± 191	$2779 \pm 656^*$	567 ± 161	0.004	829 ± 123	$2469 \pm 689^*$	474 ± 72	0.007

¹ All values are means \pm SDs. *Significantly different from baseline, $P < 0.05$ (ANOVA for repeated measures, considering time intervals and drug type as factors, with a Bonferroni post hoc multiple comparison test). [†]Significantly different from normotensive subjects, $P < 0.05$ (Student's *t* test on log-transformed variables).

² Represents the time-drug interaction.

³ FRAP, ferric-reducing antioxidant power.

atherosclerotic rabbits (31). The ability of vitamin C to cross the blood-brain barrier (22) may also justify the origin in the central nervous system of the observed restoration of baroreflex sensitivity.

Effect of vitamin C on blood pressure

Another interesting finding of the current study was that only in hypertensive patients was vitamin C administration confirmed to significantly reduce BP (32). This effect was mainly attributed to direct vascular mechanisms, such as restoration of nitric oxide-mediated vasodilation (33) by the reactive oxygen species-scavenging action of vitamin C, protecting nitric oxide from inactivation. However, the possibility exists that cardiovascular sympatho-inhibition might contribute to the BP-lowering ability shown by vitamin C. When we tested a vasodilator such as sodium nitroprusside, acting directly on smooth muscle cells, we observed that, despite a similar BP reduction, it elicited an increase in SNS activity caused by the expected baroreflex activation (2). Thus, it is conceivable that the observed decrease in sympathetic outflow after vitamin C could also contribute to BP reduction. Note that, under vitamin C administration, the reduction in BP occurred earlier than did the reduction in MSNA. A possible explanation for the timing discrepancy was that the acute BP reduction induced by direct vasodilation could have caused a baroreflex-mediated SNS activation, masking the direct sympatho-inhibitory effect in the early phase during and after vitamin C infusion. Recent experimental evidence shows that antioxidants can inhibit SNS activity at a distal level from our site of recording, possibly on neuroeffector junctions (34), which suggests another possible explanation for the earlier BP-lowering effect of vitamin C in comparison with MSNA reduction.

Conclusions

The current study supports the hypothesis that increased oxidative stress may contribute to the pathogenesis of sympathetic overactivity in essential hypertension and suggests a novel therapeutic strategy to counteract one of the main mechanisms of development and maintenance of hypertension and target organ damage. In addition, the current results confirm the role of oxidative stress as a major pathogenetic mechanism in the development of atherosclerotic and cardiovascular disease (3). It is unlikely that currently available oral antioxidants are able to achieve sympatho-inhibition, because intravenous administration of vitamin C produces substantially higher plasma concentrations than those achievable with oral administration (35). Thus, further studies are required to translate these results into clinical practice.

The authors' responsibilities were as follows—RMB, ED, and ST: designed the research; RMB, ED, MV, and IR: conducted the research; RMB, MV, and LG: analyzed the data; MV, CP, and IS: provided the essential materials; RMB and IS: wrote the manuscript; and LG, ST, and ME: revised the manuscript and contributed substantially to the discussion. None of the authors had a conflict of interest to declare.

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