Contribution of Nitric Oxide to Arterial Pressure and Heart Rate Variability in Rats Submitted to High-Sodium Intake

Silvia Lacchini, Elton L. Ferlin, Ruy S. Moraes, Jorge P. Ribeiro, Maria Claudia Irigoyen

Abstract—The aim of this study was to determine the contribution of NO to arterial pressure and heart rate variability in normotensive rats subjected to high sodium intake. Arterial pressure, heart rate, and arterial pressure and heart rate variability, baroreflex sensitivity, and pressure responsiveness were measured in male Wistar rats treated for 6 weeks (control and high sodium [1%] intake groups), before and after acute NO synthesis blockade. After treatment, no changes were observed in arterial pressure or heart rate. Arterial pressure variability was increased after sodium intake; however, heart rate variability and baroreflex sensitivity were not modified in high-sodium rats. NO synthase blockade increased arterial pressure in both groups but was higher in the high-sodium group (from 110±5 to 162±1.5 mm Hg) compared with the control group (from 109±6.7 to 144±10 mm Hg). The increase in arterial pressure was accompanied by a decrease in heart rate (from 354±28 to 303±25 bpm in control rats and from 380±34 to 298±30 bpm in high-sodium rats). NO synthase blockade increased the tachycardic response to sodium nitroprusside in high-sodium rats. Arterial pressure variability, evaluated by a nonlinear method (3D return maps), showed a larger reduction in response to NO synthase inhibition in the high-sodium group (from 162±26 to 34.8±8.6 for general index of beat-to-beat blood pressure variability) than in the control group (from 38±9.6 to 36±4.7 for general index of beat-to-beat blood pressure variability). Heart rate variability, evaluated by the SD of the R–R intervals, was not changed in control rats but was increased by NO synthase inhibition in the high-sodium rats (from 9.5±0.2 to 21.9±1.7 milliseconds). These findings suggest an important role for increased NO production in adaptation to high-sodium intake. The increase in NO system sensitivity in high-sodium intake may contribute to changes in the autonomic nervous system regulating heart rate and, especially, arterial pressure variability. (Hypertension. 2001;38:326-331.)

Key Words: blood pressure ■ heart rate ■ sodium intake ■ baroreceptors ■ nitric oxide

The regulation of arterial pressure (AP) requires the maintenance of a balance among different mechanisms of control ensuring that AP, although being continuously perturbed, always displays the tendency to come back toward a set reference point. Several mechanisms may be involved in changing AP, such as behavioral influences, neural factors, arterial baroreflex, circadian rhythm, age, and sensitivity to sodium intake, depending on different factors released or neurohormonal adjustments. The interaction between these regulatory mechanisms results in complex beat-to-beat fluctuations in AP and heart rate (HR) with nonlinear characteristics. AP and HR time-series analyses, by linear and nonlinear methods, are rich sources of information about cardiovascular control. The relationship between AP and dietary salt intake is complex and often obscured by the added influence of other factors. It has been demonstrated that the endothelium plays a fundamental role in determining vascular tone and mediates smooth muscle relaxation through NO production from the L-arginine pathway. Evidence suggesting that sodium intake increases NO synthesis by the endothelium has been accumulated. Furthermore, Lahera et al demonstrated that acute intravenous infusion of NO-nitro-L-arginine methyl ester (L-NAME) in rats decreases renal excretion of sodium and water in the absence of changes in mean AP (MAP) or in renal hemodynamics. According to these experiments, Salazar et al suggested that a decrease in NO generation by NO synthesis blockade during a high-sodium diet produces sodium retention, which might lead to volume-dependent hypertension. Because the mechanism of NO action was not completely understood, NO had been postulated to be a modulator of sympathetic nerve activity, decreasing peripheral sympathetic nerve activity. The action of NO on baroreflex control of HR modulation is still controversial but may be dependent on other factors, such as neural pathways, or physiopathological conditions. These findings are in accordance with the paradigm that blood
pressure variability is not the direct result of unbuffered variations in sympathetic discharge but, rather, is produced by an interaction between neural and humoral components. The purpose of the present experiment was to understand how endogenous formation of NO contributes to AP and HR variability (APV and HRV, respectively) and baroreceptor sensitivity in normotensive rats and whether it participates in cardiovascular regulation in those on a high-sodium diet.

Methods

Experimental Design
Male Wistar rats were obtained from the animal care unit of Federal University of Rio Grande do Sul, Porto Alegre, Brazil. All of the experiments followed the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985). We performed experiments on 21-day-old rats that were treated for 6 weeks: the control group (n=6) received filtered water, and the saline group (high-salt group, n=6) received 1% NaCl solution to drink. Both groups received a normal amount of NaCl in the food. At the end of the 6-week treatment, catheters were placed in the rats for measure AP and baroreflex sensitivity. On the next day, hypertension was induced by NO synthesis inhibition, and the same measurements were made. NO synthesis was inhibited by using the NO synthase (NOS) inhibitor Nω-nitro-L-arginine (L-NA) administered intravenously as a bolus (10 mg/kg) and additionally infused (10 mg/kg per hour) 15 minutes before and during the recording time period, respectively. The AP of each rat was measured twice, ie, before NO synthesis inhibition and after NO synthesis inhibition by L-NA.

AP Measurements
For direct measurement of AP, femoral artery and vein polyethylene catheters were inserted into the rats for AP recording and drug injection, respectively. The operation was performed 24 hours before recordings were taken in the ethyl ether–anesthetized rats. Measurements were taken over a period of 30 minutes, after a 15-minute period of resting. For AP recording, the arterial cannula was connected to a strain-gauge transducer (P23Db, Gould-Statham) coupled to a signal amplifier (HP 8805C) and to an analog-to-digital converter CODAS (2 kHz, AT/MCA CODAS, DATAQ Instruments, Inc) connected to a PC computer. During AP recording, rats were allowed to move freely.

Baroreflex Sensitivity and AP Responsiveness Measurements
Baroreflex sensitivity was measured by using increasing doses of phenylephrine (Phe, at 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 μg/mL) and sodium nitroprusside (SNP, at 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 μg/mL) administered sequentially (0.1 mL each in bolus injection) but randomly for Phe and SNP. The maximum changes in MAP and HR were measured, and baroreflex sensitivity was determined as the slope of the MAP/HR ratio (bpm/mm Hg). The AP responsiveness to intravenous SNP and Phe was also analyzed. A time interval between doses was necessary for the blood pressure to return to baseline, and administration of drugs was discontinued whenever arrhythmias were observed. The pressure and vasodepressor responses were evaluated by the slope of the regression line relating the doses of Phe and SNP to the changes in MAP, respectively. These procedures were performed at the control period and during the acute inhibition of NO synthesis.

APV and HRV
APV was analyzed by using the SD of the AP time series and by a nonlinear method called the 3D return map. This method was described for Moraes et al and has been previously used to analyze changes in APV not detected by SD in streptozotocin-induced diabetic rats treated with insulin. Briefly, 3D return maps were constructed by plotting the mean AP for each beat (APn) versus the difference between adjacent AP values [(APn−1)−(APn)] versus counts. The 3D image generated was quantified by 3 indices (P1, P2, P3) (Figure 1), which were defined as directly correlated to beat-to-beat variability and expressed in arbitrary units. The product of P1 · P2 · P3 · 10−3 was calculated as the general index, called MN, of beat-to-beat blood pressure variability. HRV was calculated by the SD of all R-R intervals.

Electrolyte Measurements
Twenty-four hours before the first pressure recording, blood (500 μL) was collected through the arterial catheters for plasma sodium, potassium, and chloride measurements. All electrolytes were measured by using the automated Lyteining System 31 (Up Super System, Abbott Laboratories) and are expressed as milliequivalents per liter.
Hemodynamic Responses to Normal and High Sodium Intake Before and After NO Synthesis Blockade

<table>
<thead>
<tr>
<th>Hemodynamic Parameters</th>
<th>Before NO Inhibition</th>
<th>After NO Inhibition</th>
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<tbody>
<tr>
<td>Control</td>
<td>High Sodium</td>
<td>Control</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>109±2.7</td>
<td>110±2</td>
</tr>
<tr>
<td>MAP variability, SD</td>
<td>5.99±1.79</td>
<td>5.93±1.52</td>
</tr>
<tr>
<td>MAP variability, MN index</td>
<td>58±9.6</td>
<td>162±26*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>354±11.4</td>
<td>380±13.9</td>
</tr>
<tr>
<td>HR variability, SD of R-R interval</td>
<td>13.1±1.8</td>
<td>9.5±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05 for control vs high sodium intake groups; †P<0.05 for within-group differences before and after NO synthesis blockade; and ‡P<0.05 for differences between control and high sodium intake groups after NO synthesis blockade.

Results

High Sodium Intake Treatment

After 6 weeks of the high-sodium diet, no significant changes occurred in body weight (314±20 g for control group versus 316±27 g for high-sodium group), AP, or HR of treated rats compared with the control rats (Table). Moreover, baroreflex responses after administration of Phe and SNP, as well as AP responsiveness, were similar in both groups (Figure 2). When evaluated by the SD, APV was not changed (5.29±2.17 versus 5.99±1.79 in control group), but APV was increased in high-sodium–fed rats compared with control rats (58±23.5 versus 162±64 arbitrary indices of MN, respectively) when evaluation was by the 3D return map. No changes in HRV evaluated by the SD and evaluated by the 3D return map were observed between the control and high-sodium groups (Table). Respective values for the high-sodium group versus control group were as follows: plasma sodium (133±1 versus 131±2 mEq/L), potassium (4.9±0.8 versus 4.1±1.0 mEq/L),

Figure 2. A and B, Vascular responsiveness and baroreceptor reflex index in control and high-sodium groups are shown. Before NO synthesis blockade, vascular responsiveness (A) and baroreflex response (B) to Phe and SNP were similar between control and high-sodium groups. C and D, After NO blockade, vascular responsiveness (C) to SNP was increased, whereas reflex tachycardia induced by SNP (D) was decreased in high-sodium rats. The numbers 1 through 8 along the abscissa indicate the sequential doses of Phe and SNP. *P<0.05 for control vs high-sodium groups; †P<0.05 for within-group differences before and after NO synthesis blockade.
and chloride (100±1 versus 100±1 mEq/L). These concentrations were similar in both groups after the 6 weeks of treatment, indicating that treatment did not modify the internal electrolyte regulation.

**NOS Inhibition**

After acute NOS blockade, both groups showed an increase in AP and a decrease in HR (Table). However, the resting AP after blockade of NOS was significantly greater in the high-NaCl diet group than in the control group (162±1.5 versus 144±10 mm Hg, respectively). After NOS blockade, pressure responsiveness was decreased in both control and high-sodium groups (Figure 2A and 2C). As can be seen in Figure 2C, NOS blockade increased the vasodepressor response to SNP in the high-sodium group. Reflex bradycardia did not change after NOS blockade in both groups. Only reflex tachycardia in response to SNP in the high-NaCl diet was significantly decreased when NO synthesis was inhibited (Figure 2D). The APV evaluated by the SD was not changed. When APV was evaluated by the MN index, derived from the 3D return map, the reduction in MAP variability after NOS blockade in the control group was not statistically significant (58±9.6 versus 36±4.7 MN index), but it was statistically significant in the high-sodium group (162±26 versus 34.8±8.6 MN index; representative samples are shown in Figure 3). After NO blockade, HRV, calculated from the SD of the R-R interval, did not change in the control group, whereas it was increased in the high-sodium group (Table).

**Discussion**

**High-Sodium Treatment**

Different mechanisms, including altered renal response and changes in peripheral neural and central endocrine mechanisms, may be responsible for the hemodynamic changes induced by sodium.16 It has been demonstrated that NO synthesis contributes to the ability of the kidney to respond hormonally to increments in sodium intake6 by this action on renal hemodynamics, sodium excretion, and renin secretion.16 Moreover, it has been demonstrated that high sodium intake leads to alterations in renal response mediated by neuronal NOS activity and macula densa function.17 As we observed, the 6-week treatment with a high sodium intake did not change AP and HR in normotensive conscious rats, which is in agreement with the experiments discussed above. The plasma electrolyte concentrations obtained after high-sodium treatment are in accordance with the well-known concept that the increase in sodium intake increases renal sodium excretion, leading to the maintenance of normal body fluid volume and AP.1

Baroreflex sensitivity was similar in sodium and control groups. The central pathways involved in the modulation of baroreflex are complex, and some substances modulate its functioning. In addition to the nucleus tractus solitarius, other areas of the brain stem and hypothalamus can modulate baroreflex function. According to recent studies,6 NO can influence baroreflex function at several of these sites of the baroreflex pathway. The finding that baroreflex sensitivity was not changed after high sodium intake is in accordance with the findings of Hogan et al,18 which showed that exogenous NO did not affect baroreflex sensitivity in humans. The findings of APV and HRV modulation in a high-sodium diet are controversial and not well understood. In the present study, however, APV calculated by the SD was unchanged; we found an increased APV calculated by the 3D return map, without modifications in baroreflex sensitivity. The APV observed in the different patterns of beat-to-beat behavior, obtained with the 3D return map (Figure 3), was not evident when it was expressed by the SD, possibly because when the SD is calculated, it considers only isolated AP values, independent of its position in the time series. Differently, the 3D return map MN index is influenced by 3 different aspects of APV: degree of concentration of AP values ($P_3$), range of AP modulation ($P_2$), and maximum AP beat-to-beat variability ($P_3$). In the representative examples of the 3D return map in Figure 3, the small APV observed in the control group (map 1a and contour curve 1b) contrasts with the evident increased beat-to-beat APV secondary to the high-sodium intake (map 3a and contour curve 3b). It resulted in a reduction in the peak concentration of points ($P_3$) and an increase in the maximum longitudinal ($P_3$) and transversal ($P_3$) axes. Acute NO synthesis blockade promoted a further reduction in APV in the control group (map 2a and contour curve 2b), increasing the peak concentration of points and reducing the longitudinal axis of the distribution. In the high-sodium group (map 4a and contour curve 4b), all 3 aspects of the 3D return map were reduced.

These findings agree with the results of Schmedtje et al,19 who found a higher APV in subjects on a high sodium intake, probably related to changes in renin-angiotensin system (RAS) activity.

After treatment, we did not find significant differences in HRV, probably because the modulation of the autonomic nervous system is different in salt-sensitive and non–salt-sensitive individuals.

**NO Synthesis Inhibition**

Several models of NOS inhibition using L-arginine analogues have been described. To study the acute systemic blockade of NOS, we used a high dose of L-NA. Acute20 NOS blockade induces hypertension similar to that observed in the present study after L-NA injection both in control rats and in high-sodium rats. Interestingly, the increase in AP was higher in high-sodium rats. This finding agrees with the results reported by Salazar et al,8 who showed that mild NOS inhibition induces an increase in AP in animals receiving a high-salt diet but not in animals receiving a normal-salt diet.

As previously demonstrated by Gardiner et al,20 acute L-NA–induced hypertension was accompanied by bradycardia. Also, these results are in agreement with the study of Castellano et al,10 which showed an increased AP and a reduced HR due to acute NOS inhibition. In the present study, the data showed that neither reflex bradycardia nor responsiveness to Phe were different between groups. On the other hand, the decrease of the tachycardic reflex response after NOS inhibition observed in the high-sodium group may be due to a higher level of AP, probably accompanied by reduced sympathetic activity, as demonstrated by Castellano et al. The vasodilatory response to SNP in high-sodium rats...
was increased probably by changes in vasodilatory mechanisms that are more intensely dependent on NO synthesis in this group.

The increased APV observed in sodium-treated rats could be due to an enhanced NO production, because during NO blockade, APV decreased in high-sodium rats, reaching values similar to those obtained in control rats under the same experimental conditions. Similar to our results, the results of Gouedard et al., who studied the effect of the angiotensin II type 1 receptor antagonist losartan and of NOS blocker L-NAME on APV in conscious rats, showed that the blockade of RAS increases a mind frequency component (0.2 to 0.6 Hz) of APV, which is decreased after NOS inhibition. These authors suggested that NO might counterbalance the APV oscillations provoked by the activation of RAS. Our results are similar to the data obtained by Castellano et al., who showed that NO synthesis inhibition reduces APV in healthy volunteers. On the other hand, Nafz et al. proposed that the NO system is a potent buffer of spontaneous AP oscillations and is most efficient in buffering frequencies with a 0.2- to 0.6-Hz range. The controversial results are probably due to the analysis system used for each experiment. The study of variability using spectral analysis evaluates different ranges of spectral frequency. The study of Nafz et al showed increased variability after NOS blockade analyzing a unique frequency range (0.2 to 0.6 Hz); however, this did not determine that all ranges of variability respond in the same manner. The system used in our experiment analyzes AP
variations in the time domain, including all ranges of frequency and permitting the visualization the entire phenomenon. Because of this, it is not possible to directly compare our results with the results obtained in the study of Nafz et al. The increase in HRV obtained after NO synthesis blockade in the high-sodium group agrees with data reported by Cordero et al., who showed an increase of R-R interval and a decrease in APV. NO has been suggested to be a mediator of cardioinhibitory mechanisms, probably increasing parasympathetic nervous activity. It is important to consider that the vagal-sympathetic effect as an index of sympathovagal balance can be evaluated by the study of the R-R interval, and the sympathovagal balance of cardiovascular regulation is the major determinant of APV. Therefore, the increase in HRV in the high-sodium group could be associated with these changes in parasympathetic activity.

The present study identifies the important role of endogenous NO synthesis in the mechanism of cardiovascular control in high sodium intake, without modifications in basal AP or HR. These findings suggest a determinant role for NO production in adaptation to high sodium intake, probably associated with RAS inhibition in the regulation of APV. In the present study, we found an enhanced increase in AP, a major bradycardia, a decrease in tachycardic reflex control of HR, and an increase in HRV in the high-sodium group in response to NOS blockade. Still, we found a decrease in APV measured by a 3D return map in the high-sodium group, and this may indicate a different sympathetic/parasympathetic participation in the control of AP. These results, taken together, suggest an increase in the endogenous NO in the high-sodium group as a mechanism of adaptation to sodium intake leading to a higher APV.

References
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