L-Arginine Restores the Effect of Ouabain on Baroreceptor Activity and Prevents Hypertension

Glaucia R. Abreu, Henrique A. Futuro-Neto, Antonio M. Cabral, Elisardo C. Vasquez

Abstract—In spontaneously hypertensive rats, ouabain exerts an excitatory effect on baroreceptor nerve activity (BNA). The aim of this study was to determine the effects of ouabain on BNA in other experimental models of hypertension and its interaction with nitric oxide. Rats were made hypertensive using the procedures for $N^\omega$-nitro-L-arginine methyl ester (L-NAME), deoxycorticosterone acetate (DOCA) salt, and 2-kidney, 1 clip (2K1C) hypertension models. In these groups, systolic arterial pressure was 195±7, 149±6, and 148±4 mm Hg, respectively, compared with 110±4 mm Hg in normotensive rats. Acute ouabain administration had an excitatory effect on BNA in normotensive rats (37±4%), an inhibitory effect in L-NAME hypertensive rats (−60±7%), and no effect in DOCA-salt and 2K1C hypertensive rats. The effects of ouabain were not related to arterial pressure levels, and no excitatory effect on BNA was observed in prehypertensive DOCA-salt rats. Long-term administration of L-arginine (3 g ⋅ kg$^{-1}$ ⋅ day$^{-1}$) prevented DOCA-salt (121±8 mm Hg) and 2K1C (104±4 mm Hg) hypertension, markedly attenuated L-NAME (130±9 mm Hg) hypertension, and restored the excitatory effect of ouabain on BNA in these groups to levels similar to the normotensive rats and their respective control groups. We conclude that ouabain has a diverse effect on BNA in experimental models of hypertension, and it can be normalized by L-arginine. The data also indicate that nitric oxide may play a pivotal role in mediating the excitatory effect of ouabain on BNA, and we speculate that a therapeutic combination of ouabain and L-arginine may be beneficial in secondary hypertension. (Hypertension. 1999;34[part 2]:729-732.)

Key Words: arginine ■ ouabain ■ L-NAME ■ baroreceptors ■ hypertension

The baroreflex acts to impede short-term fluctuations in arterial pressure (AP), but it is impaired in pathological states such as long-term hypertension. Although mechanical deformation of the nerve endings is considered the primary mechanism of baroreceptor activation, baroreceptor sensitivity is modulated by endothelial factors, such as nitric oxide (NO), which is known to inhibit baroreceptor nerve activity (BNA). However, ionic mechanisms, including the inhibition of Na$^+$-K$^+$-ATPase pump activity, increase BNA. Ouabain, which is known as an inhibitor of Na$^+$-K$^+$-ATPase pump activity, reportedly has an excitatory effect on BNA.

Recently, we reported that the excitatory effect of ouabain on BNA is increased in spontaneously hypertensive rats. The purpose of the present study was to determine whether ouabain has an excitatory effect on BNA in deoxycorticosterone acetate (DOCA) salt and 2-kidney, 1 clip (2K1C) hypertensive rats. Because we did not observe such an effect in these models and because the high plasma volume could elicit greater vascular shear stress, which is a stimulus for NO, we tried to restore the excitatory effect of ouabain on BNA in these models of hypertension by treating the animals with L-arginine long term. Additionally, we evaluated the effects of ouabain on BNA in $N^\omega$-nitro-L-arginine methyl ester (L-NAME) hypertensive rats.

Methods

Animal

The experimental protocols were performed in male Wistar rats. All experiments were conducted in compliance with the guide for biomedical research, as stated by the Brazilian Societies of Experimental Biology, and with the guiding principles of other physiological societies for research involving animals.

DOCA-Salt Hypertension

As previously described, 45-day-old rats were anesthetized with urethane (1.2 mg/kg IP) and uninephrectomized; 4 days later, they were treated with either DOCA (Sigma; 8 mg/kg SC) or vehicle (soybean oil, 0.25 mL/rat SC; n=8). DOCA was administered twice a week for 20 days (prehypertensive group; n=8) or 40 days (hypertensive group; n=8). DOCA-treated rats were allowed free access to water containing 1% NaCl and 0.03% KCl. Control animals were treated with DOCA and allowed free access to water without the electrolyte mixture (DOCA-water; n=8).

L-NAME–Induced Hypertension

This model of hypertension was induced as previously described. Briefly, rats (250 to 300 g; n=8) were housed in individual cages and treated with L-NAME (Sigma) dissolved in water (0.5 mg/mL; 11±2 mg/day) for 2 days. Each age- and body weight–matched control rat (n=8) was given water only (volume equal to that consumed by the respective hypertensive paired rat).
TABLE 1. Effects of Ouabain and L-Arginine on Basal Arterial AP Pressure in Normotensive and Hypertensive Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basal Values</th>
<th>After Ouabain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>Normotensive rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>110±4</td>
<td>76±2</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>110±2</td>
<td>80±2</td>
</tr>
<tr>
<td>L-NAME hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle-treated control</td>
<td>113±4</td>
<td>80±4</td>
</tr>
<tr>
<td>L-NAME</td>
<td>195±7</td>
<td>154±3</td>
</tr>
<tr>
<td>L-NAME+l-arginine</td>
<td>130±9†</td>
<td>105±12†</td>
</tr>
<tr>
<td>DOCA-salt hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 20 days (DOCA-water)</td>
<td>112±4</td>
<td>70±3</td>
</tr>
<tr>
<td>DOCA-salt 20 days</td>
<td>113±4</td>
<td>70±3</td>
</tr>
<tr>
<td>DOCA-salt 20 days + l-arginine</td>
<td>101±6</td>
<td>83±9</td>
</tr>
<tr>
<td>Control 40 days (DOCA-water)</td>
<td>112±6</td>
<td>68±3</td>
</tr>
<tr>
<td>DOCA-salt 40 days</td>
<td>149±6</td>
<td>92±6†</td>
</tr>
<tr>
<td>DOCA-salt 40 days + l-arginine</td>
<td>121±8†</td>
<td>76±5†</td>
</tr>
<tr>
<td>2K1C hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-operated control</td>
<td>121±4</td>
<td>78±4</td>
</tr>
<tr>
<td>2K1C</td>
<td>148±4</td>
<td>95±3†</td>
</tr>
<tr>
<td>2K1C + l-arginine</td>
<td>104±4†</td>
<td>74±3†</td>
</tr>
</tbody>
</table>

Doses of the drugs were as follows: Ouabain, 30 μg/rat IV; l-arginine, 3 g · kg⁻¹ · day⁻¹, in drinking water. *P<0.05 vs the respective control group. †P<0.05 vs the respective nontreated hypertension group.

Renovascular Hypertension

2K1C renovascular hypertension was obtained by applying a silver clip with an internal diameter of 0.2 mm to the left renal artery in 45-day-old rats, under ether anesthesia, as previously described. Control sham-operated rats were subjected to isolation, but not constriction, of the renal artery. Both 2K1C (n=8) and control (n=8) animals were studied 30 days later.

L-Arginine Treatment

Age-matched animals (n=8 per group) subjected to DOCA-salt (20 and 40 days), 2K1C, and L-NAME hypertension were simultaneously treated with the NO precursor L-arginine (Sigma) dissolved in water (3 g · kg⁻¹ · day⁻¹). The respective control groups (n=8 per group) were given water only.

Surgical Procedures for Baroreceptor Nerve Recording

After a ventral midline neck incision, the paratracheal muscles were retracted, and a tracheotomy was performed to permit the animals to breathe freely. The recurrent laryngeal nerve identified near the trachea was chosen because it displays a markedly afferent BNA. After the confirmation of whole-nerve activity, the ipsilateral vagus nerve was sectioned proximally to eliminate any respiratory-related activity on the recurrent laryngeal nerve. Pulsatile AP was recorded through a polyethylene cannula inserted into the femoral artery and connected to a pressure transducer. The femoral vein was cannulated for drug injections. The recurrent laryngeal nerve was isolated and placed on a silver bipolar electrode. Extracellular action potentials were recorded with an AC amplifier (NL 104, NeuroLog, Digitimer). The amplified signals were filtered, connected to an audio amplifier, displayed on an oscilloscope (Tektronix), and stored for subsequent analysis (Biopac Inc). The data were processed with a spike trigger and a ratemeter, and they were displayed on polygraph (Gould). All data were digitized and stored for further analysis.

Experimental Protocol

After the preparation was stabilized for 30 minutes, BNA and AP were recorded under basal conditions for 15 minutes. Next, the functional integrity of the baroreceptor nerve afferent was tested by recording the changes in BNA during increases and decreases of AP induced by phenylephrine (1 μg/kg IV) and sodium nitroprusside (0.1 μg/kg IV), respectively. After recovering the basal values of BNA and AP, ouabain (30 μg IV) was injected, and the changes in systolic AP and BNA were quantified 30 minutes later.

Statistical Analysis

Systolic AP and BNA baseline values after ouabain administration were compared among groups by use of a 2-factor ANOVA followed by a Tukey’s post-hoc test. Differences were considered significant at P<0.05.

Results

Effects of Long-Term Administration of L-Arginine on AP

Table 1 shows the baseline values of systolic and diastolic AP in hypertensive rats and their respective controls. Administration of L-NAME for 2 days caused the highest levels of both systolic and diastolic AP compared with other models of hypertension. Moderate hypertension was observed in rats treated with DOCA-salt for 40 days, but not in those treated for 20 days only, when compared with DOCA-water control.
rats. The 2K1C group showed moderate hypertension when compared with the control group. L-arginine treatment did not have any effect on AP in control rats or in rats treated with DOCA-salt for 20 days (prehypertensive). However, L-arginine treatment for 40 days prevented hypertension in 2K1C and in DOCA-salt 40-day rats. Rats treated with L-NAME plus L-arginine for 2 days showed marked attenuation (P<0.01) in the development of hypertension.

Effects of Ouabain on AP and BNA
Ouabain did not change the baseline values of systolic or diastolic AP in normotensive, 2K1C, and L-NAME hypertensive rats (Table 1). As illustrated in Figure 1 and summarized in Figure 2, after ouabain injection, a similar increase in BNA occurred in both normotensive (37±4%) and control rats: DOCA-water for 20 days (38±10%), DOCA-water for 40 days (39±10%), sham-operated 2K1C (38±5%), and L-NAME vehicle-treated (36±4%). No excitatory effect on BNA was observed with the short-term administration of ouabain in 2K1C (2±3%) or DOCA-salt for 40 days (2±10%) hypertensive rats or DOCA-salt for 20 days (−5±9%) normotensive rats (Figure 2B). In contrast to normotensive rats (37±4%) and the hypertension models, ouabain had an inhibitory effect on BNA in L-NAME rats (−60±7%). As summarized in Figure 2B, long-term treatment of hypertensive rats with L-arginine prevented ouabain’s inhibitory effect on BNA in DOCA-salt for 40 days (48±7%), 2K1C (42±5%), and L-NAME (15±8%) hypertensive animals and in DOCA-salt for 20 days prehypertensive animals (37±7%). The excitatory effect of ouabain on BNA in these groups treated with L-arginine was similar to that observed in control groups (Figure 2).

Discussion
The present study had 3 major findings: (1) ouabain inhibits BNA in L-NAME hypertensive rats but has no effect in DOCA-salt and 2K1C hypertensive rats; (2) prehypertensive DOCA-salt rats do not show the ouabain-induced excitatory effect on BNA, suggesting that such an effect is not related to AP levels; and (3) long-term administration of L-arginine prevents hypertension and restores the excitatory effect of ouabain on BNA.

In contrast to our previous observations in spontaneously hypertensive rats, the present study, we observed that ouabain injection did not increase BNA in DOCA-salt and 2K1C hypertensive rats. A reasonable explanation for the enhanced response of spontaneously hypertensive rats to ouabain could be an active mechanism of production of...
ouabain-like substances in this strain. However, high levels of ouabain have also been observed in DOCA-salt hypertensive rats, and we did not observe an excitatory effect of ouabain on BNA in this model at either the prehypertensive (20 days) or hypertensive (40 days) stage. This could not be attributed to DOCA alone because ouabain, when injected into DOCA-water rats, increased BNA to the same levels observed in normotensive rats. Interestingly, the excitatory effect of ouabain on BNA in the control groups and the lack of effect in 2K1C and DOCA-salt groups was pressure-independent; no changes in resting AP were observed in those groups.

Hypertension in long-term 2K1C rats has been attributed to a combination of elevated angiotensin II and high plasma volume, and the latter factor is a determinant in the DOCA-salt model as well. Thus, one could hypothesize that in these models of hypertension, greater vascular shear stress, which is a stimulus for endothelium-derived factors, increased the synthesis of NO, which is known to suppress the mechanism of interaction of NO with ouabain, we speculate that ouabain, in addition to its direct cardiotoxic effect, reduces efferent sympathetic activity through the activation of BNA.

References
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Hypertension. 1999;34:729-732
doi: 10.1161/01.HYP.34.4.729

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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