Interaction Between Nitric Oxide and Mineralocorticoids in the Long-Term Control of Blood Pressure

Gracia Alvarez, Antonio Osuna, Rosemary Wangensteen, Félix Vargas

Abstract—We analyzed the effects of a possible interaction between nitric oxide deficiency and mineralocorticoids on the long-term control of blood pressure and renal and endocrine variables. Six groups of uninephrectomized male Wistar rats were used: control animals and rats that received (1) N^G-nitro-L-arginine methyl ester (L-NAME) subpressor (0.5 mg/100 mL drinking fluid), (2) L-NAME pressor (35 mg/100 mL drinking fluid), (3) deoxycorticosterone acetate (DOCA; 12.5 mg/wk per rat), (4) DOCA plus L-NAME subpressor, or (5) L-NAME pressor plus DOCA. For all groups, the drinking fluid was tap water or 1% NaCl solution. We measured the time course of tail systolic blood pressure (SBP) and body weight for 3 weeks in all rats. At the end of the experimental period, we measured mean arterial pressure (direct recording) and endocrine and renal variables. Tail SBP rose significantly in the DOCA plus L-NAME subpressor–treated group but remained at normotensive levels in the DOCA-treated group. The addition of L-NAME to the subpressor dose accelerated the blood pressure increase in DOCA-salt hypertensive rats. The simultaneous administration of DOCA and L-NAME increased blood pressure and mortality rates in rats that drank water or saline compared with the rats treated with L-NAME alone. The subpressor dose of L-NAME did not increase blood pressure in saline-drinking rats. We conclude that impaired NO synthesis results in increased sensitivity to the pressor effect of mineralocorticoids in the presence or absence of an increased saline intake. Hence, nitric oxide contributes to the adaptive response to mineralocorticoid excess, perhaps through the facilitation of natriuresis and, thus, control of blood pressure. (Hypertension. 2000;35:752-757.)

Key Words: L-NAME ■ deoxycorticosterone acetate ■ hypertension, mineralocorticoid ■ vasopressins ■ endothelin

The administration of the nitric oxide (NO) synthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) induces dose- and time-dependent arterial hypertension. NO is a vasodilator autacoid that plays an important role in renal hemodynamics and sodium excretion. NO is a modulator of the homeostatic response to an increase in sodium intake. Thus, it has been reported that an increase in salt intake results in enhanced NO production and that the inhibition of NO synthesis by a subpressor dose of L-NAME may result in a sodium-dependent form of hypertension in dogs. NO deficiency has been implicated in the development of hypertension in Dahl sodium-sensitive rats, and a high-salt diet aggravates the hypertension and renal injury in rats treated on a long-term basis with L-NAME. However, others have reported that long-term sodium restriction and short-term or long-term sodium loading did not affect the increase in blood pressure (BP) induced by long-term L-NAME treatment.

The long-term administration of the mineralocorticoid deoxycorticosterone acetate (DOCA) induces sodium retention, and in the presence of a high salt intake, it produces a well known volume-dependent type of hypertension. Experimental protocols in vivo and in vitro suggest that basal NO synthesis is augmented in this model of hypertension and that this increased NO synthesis is apparently a compensatory response to prevent increases in vascular resistance during the development of DOCA-salt hypertension. In contrast, the results of a study with isolated kidneys from DOCA-salt hypertensive rats showed a reduced acetylcholine-induced NO release that was improved with the oral administration of L-arginine, which did not, however, influence the time course of systolic BP (SBP) elevation in the DOCA-salt hypertensive rats.

As mentioned earlier, the importance of NO and mineralocorticoids in BP regulation and sodium homeostasis is widely recognized, but the interaction between them in the control of BP and renal function has not yet been evaluated. Therefore, in the present study, we analyzed the possible interaction between NO deficiency and mineralocorticoids on their respective pressor, renal, and endocrine effects.

Methods

Animals
One hundred twenty male Wistar rats (weight 160 to 200 g) that were born and raised in the animalario of the University of Granada were used in the present study. All experiments were performed according to the guidelines for the ethical care of animals of the European Union. Rats were randomly assigned to 1 of 6 experimental groups: control animals (CONT), DOCA treatment (DOCA), L-NAME...
treatment at the subpressor dose (NAME-SP), DOCA plus L-NAME treatment at the subpressor dose (DOCA-NAME-SP), L-NAME treatment at the pressor dose (NAME-P), or DOCA plus L-NAME treatment at the pressor dose (DOCA-NAME-P). All animals were uninephrectomized while under ether anesthesia before the experiments started. The groups were used for 2 experiments. In experiment 1, all animals drank tap water; in experiment 2, the animals drank 1% NaCl. L-NAME was administered in the drinking fluid at a concentration of 35 mg/100 mL (pressor dose) or 0.5 mg/100 mL (subpressor dose). In the saline-drinking groups, the concentration of the NO inhibitor was adjusted according to the fluid intake. DOCA was administered subcutaneously at a dosage of 12.5 mg/wk per rat.

Experimental Protocol
Tail SBP was measured twice a week with the use of tail-cuff plethysmography in unanesthetized rats. The treatments were maintained for 3 weeks, and all rats of each group were then housed in metabolic cages with free access to food and their respective drinking fluids. After 2 days of adaptation, food and fluid intake and urine output during 24-hour periods were measured for 2 consecutive days. The values obtained on each experimental day were averaged for statistical purposes. Subsequently, a blood sample was taken from the tail to measure plasma levels of creatinine. The urinary variables that we measured were diuresis, natriuresis, creatinine, microalbuminuria, and the excretion of immunoreactive antidiuretic hormone (ADH) and endothelin (ET).

At the end of the metabolic studies, all animals were anesthetized with ethylc ether, and the right femoral artery was cannulated to obtain direct MAP measurements and blood samples. After a 24-hour recovery period, BP was continuously measured during 60 minutes in conscious rats. Values obtained during the final 30 minutes were averaged on a minute-to-minute basis to obtain the mean BP value. Blood samples were then taken; the plasma variables that were measured were sodium, potassium, and creatinine.

Analytical Procedures
Urinary ADH and ET levels were measured in extracted (C18 column) urine samples with the use of radioimmunoassay kits purchased from Amersham Iberica. Plasma and urine electrolytes, urea, and creatinine were measured on the same day in an autoanalyzer (model CX4; Beckman). Microalbuminuria was measured with nephelometry.

Statistical Analysis
The evolution of SBP with time was compared with the use of a nested design, with groups and days as fixed factors and rat as the random factor. When the overall difference was significant, Bonferroni’s method with an appropriate error was used. The remainder of the variables were compared at the end of the experiment with the use of 1-way ANOVA, and subsequent pairwise comparisons were made with the Newman-Keuls test.

Results
Blood Pressure
Figure 1 provides a summary of BP data and shows the evolution of tail SBP as measured with plethysmography (left) and the final MAP as measured with direct recording in
conscious rats (right). The addition of a subpressor dose of L-NAME to the drinking water of DOCA-treated rats (DOCA-NAME-SP) resulted in increased BP, whereas the administration of DOCA alone maintained BP at normal values (top left). Figure 1 also shows that treatment with DOCA aggravated L-NAME hypertension; BP was significantly higher in the DOCA-NAME-P group than in the NAME-P group. In rats that drank 1% NaCl, the administration of L-NAME at the subpressor dose exacerbated the course of hypertension (Figure 1, bottom left). These results were later confirmed with direct BP measurement (Figure 1, bottom right). The NAME-SP group had normal BP values. Figure 1 (bottom) also shows that DOCA-salt and L-NAME treatments had additive effects to increase BP, inducing a type of malignant hypertension with an increased mortality rate. Thus, the DOCA-NAME-P group showed elevated BP compared with both DOCA and NAME-P groups. Three rats in the DOCA-NAME-P group died during the course of treatment after 2 to 3 days of decreased motility and clear signs of illness, whereas none of the animals in the other groups died. The remaining rats of this experiment demonstrated normal behavior with no signs of illness. It is interesting that the saline-drinking NAME-P group showed an accelerated increase in BP and final MAP compared with the water-drinking NAME-P group (NAME-P/no salt 131.2±2 mm Hg, NAME-P/salt 147.2±2 mm Hg, P<0.01). Final body weight was similar in all the groups for experiment 1, although the DOCA-NAME-P group showed a tendency to gain less weight, and in experiment 2, this group, which drank saline, showed a clear reduction in body weight (DOCA-NAME-P 206±5 g, CONT 319±10 g, P<0.01)

Renal and Metabolic Variables
In rats that drank water (experiment 1), the DOCA-NAME-P group showed a significant increase in plasma levels of creatinine (0.55±0.02 mg/dL) with a concomitant reduction in glomerular filtration rate (GFR) (Table) compared with the CONT group (0.31±0.09 mg/dL). No other experimental group significantly differed from the CONT group in any variable. In experiment 2, plasma levels of creatinine (CONT 0.26±0.02, DOCA 0.55±0.03, DOCA-NAME-SP 0.55±0.06, NAME-P, 0.46±0.03, DOCA-NAME-P, 1.76±0.15 mg/dL, P<0.01 versus CONT) and GFR (Table) were increased and reduced, respectively, in all groups with elevated BP, and these variables were more affected in the DOCA-NAME-P group. Urea levels were also markedly increased in the DOCA-NAME-P group (235±37 mg/dL, CONT 36±3.5 mg/dL, P<0.01). Plasma sodium and potassium levels were significantly increased and reduced, respectively, in all DOCA-treated groups. Plasma sodium levels were increased in the NAME-P group (data not shown).

Diuresis, natriuresis, and kaliuresis were similar in control and experimental groups in experiment 1 (Table). In experiment 2, all of the DOCA-treated groups showed increased diuresis and natriuresis, with no significant changes in kaliuresis (Table). In experiment 1, microalbuminuria was significantly increased in both groups treated with L-NAME at the pressor dose (Table), whereas among the saline-drinking rats (experiment 2), microalbuminuria was greater in all of the experimental groups except for the NAME-SP group compared with the CONT group (Table).

Endocrine Variables
In water-drinking rats (experiment 1), the total excretion of immunoreactive ADH (Figure 2) was significantly increased in the 3 DOCA-treated groups and in the NAME-P group. In experiment 2, all of the DOCA-treated groups also showed increased total ADH excretion (Figure 2). In the latter experiment, the L-NAME treatment produced no increase in ADH excretion above that produced with the saline intake. The total urinary excretion of immunoreactive ET (Figure 2) was also increased in all experimental groups with elevated BP (Figure 2).
was only significantly increased in the DOCA-NAME-P group in experiment 1, whereas it was significantly increased in all of the experimental groups versus the CONT group in experiment 2.

Discussion
One of the main findings of this report is that the addition of a subpressor dose of L-NAME to DOCA-treated rats induced an increase in BP, indicating that NO may play a homeostatic role through the prevention of the pressor effect of DOCA. However, when DOCA was added to a pressor dose of L-NAME, it produced a increase in BP above that observed in the rats treated with L-NAME alone. These results suggest that L-NAME and DOCA have additive affects on sodium retention and, therefore, on increasing BP.

In the saline-drinking rats, the simultaneous administration of DOCA and L-NAME at the subpressor dose exacerbated the course of DOCA-salt hypertension. These results confirm that partial NO synthesis inhibition disturbs the homeostatic response to an excess of mineralocorticoids. Interestingly, the administration of the subpressor dose of L-NAME to uninephrectomized saline-drinking rats did not increase BP; these results are in contrast to those reported by Salazar et al in dogs and by Yamada et al in Munich-Wistar rats, who observed an increased BP after treatment with salt and a subpressor dose of L-NAME. These discrepancies may be due to the differing susceptibilities of distinct species, the strain of the rats, the dose, the route of L-NAME administration, or the sodium content in the diet. Our results also show that DOCA-salt and L-NAME treatments have additive effects to increase BP, inducing a type of severe hypertension with an increased mortality rate. Moreover, in the present study, we found that the addition of salt accelerated the course of L-NAME hypertension and resulted in a greater MAP at the end of the study. These data agree with previous reports but contrast with earlier observations by workers at our laboratory that showed an increased salt intake did not change the time course of NO-induced hypertension produced with a higher dose of L-NAME. To explain these discrepancies, it has been suggested that the NO synthase (NOS) inhibition model may follow different patterns, depending on the extent of NO inhibition. Thus, very low doses of NOS inhibitor produce a purely volume-dependent hypertension, whereas high-grade near-complete NOS inhibition promotes renal and systemic vasoconstriction that is not affected by salt intake changes.

Only the DOCA-NAME-P group in experiment 1 showed a significant increase in the plasma levels of urea and
creatinine with a concomitant reduction in GFR versus the CONT group. However, in experiment 2, all DOCA-salt hypertensive groups showed significant reductions in GFR, which was markedly reduced in the DOCA-NAME-P group. These data indicate that DOCA and L-NAME at the pressor dose have additive effects on renal dysfunction. The normal plasmatic levels of creatinine and GFR in the NAME-P group of experiment 1 concur with the results of Pollock et al.\textsuperscript{17} but contrast with observations in L-NAME hypertensive rats that were treated for a longer period of time and with greater doses of L-NAME.\textsuperscript{9} The appearance of higher levels of creatinine and reduced creatinine clearance in the experiment 2 groups indicates a greater degree of renal insufficiency in the saline-drinking groups. These data are consistent with reports by other authors\textsuperscript{8,15} that increased saline intake aggravates renal injury in L-NAME–treated hypertensive rats. In experiment 1, microalbuminuria was significantly increased in both groups treated with L-NAME at the pressor dose. These data agree with previous observations of proteinuria in L-NAME–treated hypertensive rats.\textsuperscript{16} Microalbuminuria was greater in the hypertensive groups of experiment 2, except for the DOCA-NAME-P group, which may be due to the low GFR observed in this group. This alteration, together with the important reduction in GFR reported earlier, confirms that a high-salt diet aggravates renal injury at this dose of L-NAME. This was confirmed through histological examination of the kidneys; thus, the NAME-P group of experiment 1 showed microaneurysms in the glomerular vessels, and this same group in experiment 2 (which drank saline) showed more severe alterations, such as glomerular sclerosis and collapse, hyaline arteriopathy, vascular obliteration, and fibrinoid necrosis (G.A., A.O., R.W., and F.V., unpublished observations).

We also examined the effect of the interaction between NO deficiency and DOCA on the urinary excretion of ADH and ET, because it has been reported that the two hormones may interact and play a role in DOCA-salt hypertension\textsuperscript{18,19} and that ET might participate in the renal dysfunction of L-NAME hypertension.\textsuperscript{20} Moreover, the stimulation of ET\textsubscript{B} receptors, which are abundantly present in collector tubules and inner medulla, induced diuresis and natriuresis. The diuresis is produced through inhibition of the cAMP induced by ADH, and the natriuresis is produced through inhibition of Na\textsuperscript{+},K\textsuperscript{+}-ATPase in proximal and collector tubules. Thus, rats and mice with congenital deficits in ET\textsubscript{B} receptors show an increased salt sensitivity and hypertension.\textsuperscript{20}

The results indicate that DOCA increases ADH production regardless of whether there is increased saline intake and that L-NAME hypertension courses with an increased ADH production. This variable has not to our knowledge been previously measured in chronic NO deficiency. In experiment 2, all of the DOCA-treated groups showed increased total ADH excretion, as has already been reported for this type of experimental hypertension.\textsuperscript{18} The treatment with L-NAME did not produce an additional increase in ADH excretion above that produced by the saline intake. Thus, increased saline intake seems to preclude the effect of L-NAME on ADH production. Moreover, the lack of response to the concomitant administration of L-NAME to DOCA-salt hypertensive rats may be because the ADH excretion rate would be close to the maximum due to the administration of DOCA and salt.

In experiment 1, the total urinary excretion of immunoreactive ET was only significantly elevated in the DOCA-NAME-P group, which, as reported earlier, showed important renal dysfunction. In experiment 2, the total excretion of immunoreactive ET was significantly increased in all the experimental groups compared with the control animals. These data appear to indicate that salt sensitizes the kidney to produce ET in response to mineralocorticoids or NO inhibition probably to facilitate natriuresis. The urinary excretion of immunoreactive ET in DOCA-salt hypertensive rats despite other reports of unchanged plasma levels of immunoreactive ET.\textsuperscript{19} This increased urinary ET level may be the result of an increased renal production of ET, because it has been observed that urinary ET is mainly of renal origin.\textsuperscript{21} Therefore, our findings of increased urinary excretion of immunoreactive ET in DOCA-salt hypertension may indicate an role for this peptide in inhibition of ADH activity, facilitation of natriuresis, and, therefore, contribution to the polyuria/polydipsia syndrome of these rats.

In conclusion, the results of the present work show that (1) sensitivity to the pressor effect of mineralocorticoids, in the presence or absence of an increased saline intake, is increased by an impaired NO synthesis and (2) simultaneous treatments with L-NAME and DOCA have additive effects on BP and decrease renal function regardless of an increased saline intake.

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References


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