Role of nitric oxide in modulating renal function and arterial pressure during chronic aldosterone excess


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Role of nitric oxide in modulating renal function and arterial pressure during chronic aldosterone excess. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R197–R202, 1999.—Chronic aldosterone (Aldo) excess is associated with transient sodium retention, extracellular fluid volume expansion, renal vasodilation, and hypertension. The purpose of this study was to determine the role of nitric oxide (NO) in mediating the renal vasodilation and the escape from the sodium-retaining actions of Aldo. To achieve this goal, we examined the long-term effects of Aldo (15 µg·kg⁻¹·min⁻¹ for 7 days) in conscious, chronically instrumented control dogs (n = 9) and in dogs (n = 12) pretreated with the NO synthesis inhibitor N⁶-g-nitro-L-arginine methyl ester (L-NAME; 10 µg·kg⁻¹·min⁻¹). In control dogs, Aldo caused a transient sodium retention (126 ± 6 to 56 ± 2 meq/day) followed by a return of sodium excretion to normal levels. Aldo also increased renal plasma flow by 15% (205 ± 13 to 233 ± 16 ml/min), glomerular filtration rate by 20% (72 ± 3 to 87 ± 5 ml/min), and arterial pressure from 90 ± 3 to 102 ± 3 mmHg. Aldo increased urinary nitrate/nitrite excretion by 60% in the control dogs. Although the sodium-retaining (144 ± 7 to 56 ± 7 meq/day) and arterial pressure (122 ± 6 to 136 ± 5 mmHg) responses to Aldo were the same in dogs pretreated with L-NAME compared with control, the renal hemodynamic response was markedly attenuated. The results of this study suggest that NO plays an important role in mediating the renal vasodilation during chronic Aldo excess.

IT IS WELL KNOWN that the kidney is able to “escape” from the sodium-retaining effects of mineralocorticoids despite an immutable action of the mineralocorticoid on the distal nephron to enhance sodium reabsorption (1, 3, 4, 6). Thus the continuous administration of mineralocorticoids to normal subjects induces a short period of sodium retention followed by a return to sodium balance (1, 3, 4, 6). Although it is thought that sodium delivery to the distal nephron is enhanced during mineralocorticoid excess, thereby overwhelming the capacity of the distal nephron to reabsorb sodium and leading to a normalization of sodium excretion, mechanisms mediating this effect have not yet been fully elucidated (15, 19, 21, 30).

Investigators have postulated that escape from the sodium-retaining actions of aldosterone is caused by renal and circulatory compensations activated by extracellular fluid volume expansion (7, 16, 17, 20, 30, 31). The elevation in extracellular fluid volume expansion during aldosterone excess is normally associated with significant increases in arterial pressure, renal blood flow, and glomerular filtration rate, all of which could offset the direct sodium-retaining actions of aldosterone (1, 3, 19, 20, 36). Increased levels of natriuretic factors, such as atrial natriuretic factor, kinins, and prostaglandins, as well as suppression of sodium-retaining factors, such as renal sympathetic nerve activity and angiotensin II, have also been postulated to play a role in aldosterone escape (7, 11, 16, 17, 19, 30, 31).

Another potential factor that could contribute to the renal and cardiovascular compensatory responses to chronic aldosterone excess is nitric oxide (NO). NO is synthesized in the kidney and is a potent renal vasodilator and natriuretic substance (9, 10, 27, 34, 37, 38). Several studies have also indicated that NO synthesis is increased in response to acute and chronic elevations in extracellular fluid volume (5, 16, 23, 24, 32, 33, 37). In addition, blockade of nitric oxide synthesis has been reported to blunt the natriuretic response to acute volume expansion (22, 23). Moreover, an increase in renal perfusion pressure, which has been shown to be a major contributor to the compensatory escape from mineralocorticoids, is also thought to be a stimulator of renal NO synthesis (12–14, 17, 24, 34). Although these findings suggest that NO could potentially contribute to the renal response to aldosterone excess, it is unknown whether aldosterone excess is associated with elevations in NO synthesis and, more important, whether NO plays an important role in mediating aldosterone escape. Therefore, the purpose of this study was to determine the role of NO in modulating the cardiovascular and renal hemodynamic excretory responses to chronic aldosterone excess. To achieve this objective, we examined the long-term effects of aldosterone in conscious, chronically instrumented, control dogs and in dogs pretreated with the NO synthesis inhibitor N⁶-g-nitro-L-arginine methyl ester (L-NAME).

METHODS

Animal instrumentation. This study was conducted in 21 mongrel dogs that were conditioned before the study. Surgical procedures were performed under pentobarbital sodium anesthesia (30 mg/kg iv) and under aseptic conditions (11, 32, 35). The experimental procedures were done according to the guidelines of the National Institutes of Health. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

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All animals underwent surgery for implantation of chronic vascular catheters in both femoral arteries and veins (11, 35). The catheters were tunneled subcutaneously and exteriorized in the upper back for blood sampling and infusions and to allow for continuous monitoring of arterial pressure. After at least 1 wk of recovery from surgery, all dogs were housed in individual metabolic cages in a temperature- and humidity-controlled room with a 12:12-h dark-light cycle. Isotonic saline was continuously infused intravenously by using a roller pump (Wiz Pumps, Cambridge, MA) at a rate of ~900 ml/day (140 meq NaCl). Arterial pressure was recorded through a pressure transducer that was mounted at the level of the heart. Blood pressure signals were continuously recorded, and the analog signals were sent to a digital computer to be analyzed. The computer was adjusted to take samples each minute and calculate the average mean arterial pressure and heart rate during the period from 2:00 PM to 8:00 AM. Daily care of the dogs was performed between 8:00 AM and 2:00 PM.

During the entire period of the study, dogs were placed on a sodium-deficient diet (H/D Hills Pet Products, Topeka, KS) that provided ~6–7 mmol of sodium and 65 mmol of potassium per day. In addition, dogs were supplemented with 10 ml of a multivitamin preparation (VAL syrup, Dodge Labs, Ft. Dodge, IA) each day. Sodium intake was fixed at ~145 mmol/day, which includes 138 meq from the saline infusion and 6–7 meq from the food.

Experimental protocol. The cardiovascular and renal effects of aldosterone were determined in control dogs (n = 9) and in dogs pretreated with the NO synthesis inhibitor L-NAME (n = 12). Before aldosterone infusion, a period of 1 wk was allowed to achieve stable hemodynamic measurements and a state of sodium balance. After we obtained a 1-wk period of control measurements, aldosterone was infused at a rate of 15 µg·kg⁻¹·min⁻¹ for 7 days in control dogs and in dogs pretreated with L-NAME (10 µg·kg⁻¹·min⁻¹) for at least 10 days before the beginning of the study and throughout the study. The dose of L-NAME chosen for this study was previously shown by our laboratory to produce maximum blood pressure and renal hemodynamic effects in conscious dogs (25, 26, 35). A 7-day recovery period followed the aldosterone infusion period in each group of dogs.

Arterial pressure, heart rate, and urinary excretion of sodium and potassium were measured daily. Renal hemodynamics and urinary excretion of nitrate/nitrite were determined on days 3 and 7 of the prealdosterone infusion period as well as during the first, third, and seventh days of aldosterone infusion. Glomerular filtration rate and renal plasma flow were also measured on the last day of the recovery period. Glomerular filtration rate and renal plasma flow were determined from the clearances of [¹²⁵I]iothalamate (Glofil; Isotex Diagnostics, Friendwood, TX) and [¹³¹I]iodohippurate (Hippuran; Syntex, Jackson, MS), respectively, by using the single-injection technique as previously described (19).

Analytic procedures. Urinary and serum sodium and potassium were determined by flame photometry (IL-943; Instrumentation Laboratory, Lexington, MA). Concentrations of [¹²⁵I] and [¹³¹I] in plasma were determined by a gamma counter (model 1185; Searle, Des Plaines, IL). Urinary nitrate/nitrite was determined by using Escherichia coli as the source of nitrate reductase to convert nitrate to nitrite, using sodium nitrate as the standard to verify that all nitrate is converted to nitrite (2). The concentration of nitrite was measured colorimetrically using the Griess reagent. Sodium nitrite was used as the standard, and the data were expressed as millimoles nitrate/nitrite excreted per 24 hours.

Statistics. Data are expressed as means ± SE. Comparisons of control data with the period after aldosterone were analyzed by using one-way analysis of variance for repeated measures and subsequent Dunnett’s t-test for simultaneous comparisons within groups and subsequent use of Bonferroni t-test for nonsimultaneous comparisons between groups. A P value <0.05 was accepted as statistically significant.

RESULTS

Figure 1 shows changes in mean arterial pressure and heart rate in response to a continuous intravenous infusion of aldosterone at a rate of 15 µg·kg⁻¹·min⁻¹ for 7 days in control dogs and in dogs pretreated with L-NAME. Aldosterone significantly increased mean arterial pressure and heart rate in control dogs and in dogs pretreated with L-NAME. Aldosterone in the control dogs increased mean arterial pressure during the last 3 days of infusion by ~12 mmHg, from 90 ± 3 to 102 ± 3 mmHg (P < 0.05). Mean arterial pressure in L-NAME-pretreated dogs averaged 122 ± 6 mmHg under basal conditions and increased by 14 mmHg to 136 ± 5 mmHg (P < 0.05) during the last 3 days of the aldosterone infusion period. The increases in mean arterial pressure in response to aldosterone were not significantly different between the control and L-NAME-pretreated dogs. Heart rate in the control dogs aver-
aged 53 ± 4 beats/min under basal conditions, 50 ± 5 beats/min during aldosterone infusion, and 47 ± 5 beats/min during the recovery period. Heart rate in L-NAME-pretreated dogs averaged 53 ± 4 beats/min under basal conditions, 54 ± 3 beats/min during aldosterone infusion, and 57 ± 3 beats/min during the recovery period. Heart rate between the control and L-NAME groups of dogs was not significantly different during control, experimental, and recovery periods of the protocol.

Renal plasma flow and glomerular filtration rate responses to a continuous intravenous infusion of aldosterone in control dogs and in dogs pretreated with L-NAME are shown in Fig. 2. Aldosterone infusion resulted in a significant increase in renal plasma flow and glomerular filtration rate in the control dogs. Renal plasma flow in control dogs increased by 15% (P < 0.05), from 205 ± 13 to 233 ± 16 ml/min, in response to aldosterone while glomerular filtration rate increased by 20% (P < 0.05), from 72 ± 3 to 87 ± 5 ml/min. The renal hemodynamic response to aldosterone was markedly attenuated in dogs pretreated with L-NAME. Renal plasma flow in L-NAME-pretreated dogs averaged 160 ± 9 ml/min under basal conditions and 161 ± 10 ml/min during aldosterone infusion. Glomerular filtration rate in L-NAME-pretreated dogs averaged 60 ± 4 ml/min under basal conditions and 66 ± 4 ml/min during the aldosterone infusion period.

Urinary sodium excretion and potassium excretion responses to aldosterone in control dogs and in dogs pretreated with L-NAME are depicted in Fig. 3. Sodium and potassium excretory responses to chronic aldosterone administration were similar between the control and L-NAME-treated dogs. Urinary excretion of sodium in the control dogs averaged 126 ± 6 mmol/day under basal conditions, decreased transiently to 56 ± 2 mmol/day (P < 0.05) on day 1 of aldosterone infusion, and then returned toward normal levels for the remainder of the aldosterone infusion period. Sodium excretion in the L-NAME-pretreated dogs averaged 144 ± 7 mmol/day under basal conditions, decreased to 56 ± 7 mmol/day (P < 0.05) on day 1 of aldosterone infusion, and then returned toward normal levels for the remainder of the aldosterone infusion period. The L-NAME-pretreated dogs, however, had a greater natriuretic response on day 1 after cessation of aldosterone compound than the control group. Potassium excretion in the control dogs averaged 49 ± 3 mmol/day under basal conditions, increased to 61 ± 8 mmol/day (P < 0.05) on day 1 of aldosterone infusion, and then returned toward normal levels for the remainder of the aldosterone infusion period. Potassium excretion in L-NAME-
infusion. Urinary excretion of nitrate/nitrite in the control dogs averaged 73 ± 8 mmol/day under basal condition and increased to a maximal level of 119 ± 6 mmol/day (P < 0.05) on day 3 of aldosterone infusion. In contrast, urinary excretion of nitrate/nitrite did not increase in response to aldosterone in dogs pretreated with L-NAME. Urinary excretion of nitrate/nitrite in the control dogs averaged 73 ± 8 mmol/day under basal condition and increased to a maximal level of 119 ± 6 mmol/day (P < 0.05) on day 3 of aldosterone infusion. Urinary excretion of nitrate/nitrite in the L-NAME-pretreated dogs averaged 55 ± 9 mmol/day under basal conditions and 43 ± 9 mmol/day during aldosterone infusion.

DISCUSSION

We report that chronic aldosterone excess in normal dogs produces a transient sodium retention followed by a return to normal sodium balance. This sodium excretory response to aldosterone was accompanied by significant increases in arterial pressure, renal plasma flow, glomerular filtration rate, and urinary excretion of nitrate/nitrite. To determine the role of NO in modulating the cardiovascular and renal hemodynamic excretory responses to chronic aldosterone excess, we also examined the long-term effects of aldosterone in dogs pretreated with the NO synthesis inhibitor L-NAME. Although the sodium-retaining and arterial pressure responses to aldosterone were similar in dogs pretreated with L-NAME, the renal hemodynamic responses were virtually abolished. These results suggest that NO plays an important role in mediating the renal vasodilation during chronic aldosterone excess in dogs.

The return of sodium excretion to normal levels despite a continued renal tubular action of aldosterone has been suggested to occur as a result of activation of compensatory natriuretic systems (7, 11, 16, 17, 30, 31). To determine the importance of NO in modulating the time course of escape from the sodium-retaining actions of aldosterone, the effects of aldosterone were examined in dogs pretreated with L-NAME. Infusion of aldosterone into normal dogs for 7 days resulted in a 50% reduction in sodium excretion on day 1 of the infusion period and returned to normal levels by days 2 and 3. Potassium excretion increased during the first 1–2 days of aldosterone infusion. We found that inhibition of NO synthesis did not affect the time course of aldosterone-induced sodium retention or kaliuresis. Infusion of aldosterone into dogs pretreated with L-NAME also resulted in a 50% reduction in sodium excretion on day 1 of the infusion period and returned to normal levels by days 2 and 3 of aldosterone infusion. Although potassium excretion in the L-NAME-pretreated dogs was greater than in the control dogs on day 2 of aldosterone infusion, excretion rates were quite similar between the two groups. These findings indicate that NO is not essential in causing sodium excretion to return to normal levels during chronic aldosterone excess.

Chronic infusion of aldosterone resulted in significant increases in renal plasma flow and glomerular filtration rate in control dogs. Although increases in glomerular filtration rate have been proposed to be an important mechanism whereby aldosterone escape occurs, the factors responsible for enhancing renal hemodynamics during aldosterone excess are unknown (17, 19, 20, 29, 30). Although elevations in renal perfusion pressure play a critical role in the escape from the sodium-retaining actions of aldosterone, this mechanism does not appear to mediate the renal hemodynamic response (17). The results of this study indicate that NO plays an important role in mediating the elevation in renal plasma flow and glomerular filtration rate during chronic aldosterone excess. In contrast to the control dogs, where renal plasma flow and glomerular filtration rate increased by 15–20% during aldosterone infusion, the renal hemodynamic response to chronic aldosterone excess was abolished in dogs pretreated with L-NAME. The lack of a renal hemodynamic response to aldosterone in the L-NAME-treated dogs could be due to the increased basal renal vascular resistance induced by L-NAME. This is unlikely, however, because we previously reported that increases in renal vascular resistance induced by high intrarenal levels of angiotensin did not alter the renal hemodynamic response to aldosterone (28). On the basis of our results, it appears that an increase in NO synthesis plays an important role in mediating the renal vasodilation and hyperfiltration in response to chronic aldosterone excess. Our results also indicate that increases in glomerular filtration rate and renal plasma flow in response to aldosterone are not essential for the kidney to escape from the sodium-retaining actions of aldosterone.

Fig. 4. Bar graph shows changes in urinary excretion of nitrate/nitrite in response to a continuous intravenous infusion of aldosterone at a rate of 15 µg·kg⁻¹·min⁻¹ for 7 days in control dogs and in dogs pretreated with L-NAME. Values are means ± SE.
An increase in renal perfusion pressure has been shown to play an important role in offsetting the direct sodium-retaining actions of aldosterone (17). If NO plays a role in the escape from the sodium-retaining actions of aldosterone, one would predict that inhibition of NO would not totally prevent a return of sodium excretion to normal levels during aldosterone excess but rather a higher level of arterial pressure would be required to maintain sodium balance (4, 14). For example, we previously reported that preventing reductions in angiotensin level during aldosterone infusion delayed the time course of sodium retention and increased the arterial pressure response to aldosterone (28). An important finding in this study is that inhibition of NO synthesis did not increase the level of the arterial pressure required to maintain sodium balance during chronic aldosterone excess. In control dogs, chronic aldosterone excess resulted in an increase in arterial pressure of \( \sim 12 \text{ mmHg} \). Likewise, aldosterone infusion into \( \text{L-NAME} \)-pretreated dogs increased arterial pressure by \( 14 \text{ mmHg} \). Thus NO does not appear to play a role in altering the pressure-natriuresis relationship during aldosterone excess.

Under conditions of chronic NO synthesis blockade, it appears inhibition of tubular sodium reabsorption is the predominant mechanism for escaping from the sodium-retaining action of aldosterone. The exact mechanism that mediates the inhibition of tubular sodium reabsorption during chronic aldosterone excess is unclear. Because similar increases in arterial pressure occurred in both groups it would appear that a pressure-natriuresis could not explain the differences in the fractional excretion of sodium response between the control and \( \text{L-NAME} \)-pretreated groups. However, we cannot rule out the possibility that during chronic NO synthesis inhibition the sensitivity of the renal tubules to pressure may be enhanced. One possible explanation for the enhanced sensitivity could be due to the fact that the \( \text{L-NAME} \)-pretreated group starts out at a higher baseline arterial pressure. It is well known that the pressure-natriuresis relationship is much steeper at higher levels of arterial pressure. Although the increase in arterial pressure in response to aldosterone was the same in \( \text{L-NAME} \)-pretreated dogs, the fact that they were at a steeper part of the pressure-natriuresis curve may account for the increased sensitivity to the rise in renal perfusion pressure. Another possibility is that alternative natriuresis factors such as atrial natriuretic factor or prostaglandins are activated during aldosterone excess in \( \text{L-NAME} \)-treated dogs.

To determine the long-term effects of aldosterone excess on NO production, 24-h urinary excretion of nitrate/nitrite was measured before and after aldosterone administration. In control dogs, aldosterone increased urinary excretion rate of nitrate/nitrite by \( \sim 60-65\% \) on day 3 of aldosterone infusion. This finding suggests that chronic aldosterone excess results in enhanced production of NO. Whether the increase in NO production during aldosterone excess is of renal origin cannot be ascertained in this study, because changes in 24-h urinary excretion of nitrate/nitrite only estimate changes in whole body production of NO. The fact that NO synthesis inhibition markedly attenuated the renal hemodynamic response to aldosterone, however, suggests that aldosterone excess may alter renal production of NO. Further studies are necessary to determine whether aldosterone excess stimulates renal production of NO and whether aldosterone has this effect via a direct or indirect action.

The chronic dose of \( \text{L-NAME} \) used in this study to inhibit NO synthesis was \( 10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). We previously reported that this dose of \( \text{L-NAME} \) produces a 15–20% elevation in arterial pressure associated with a 10–15% reduction in renal plasma flow in normal conscious dogs (25, 26, 34). We have also found that higher doses of \( \text{L-NAME} \) do not result in greater systemic and renal hemodynamic responses (35). Although the renal hemodynamic response to \( \text{L-NAME} \) in this study is similar to that of our previous studies, the arterial pressure response was higher than we previously reported (35). This exaggerated arterial pressure response, however, is most likely due to the higher level of sodium intake used in the current study. Our finding that \( \text{L-NAME} \) produced significantly higher levels of blood pressure in dogs on a high-salt diet is consistent with previous studies indicating that NO synthesis inhibition produces a salt-sensitive form of hypertension (5, 33, 37).

The urinary excretion rate of nitrate/nitrite before aldosterone infusion was slightly, but not significantly, lower in the \( \text{L-NAME} \) group than in the control group. The inability to totally suppress urinary excretion of nitrate/nitrite with \( \text{L-NAME} \) is not surprising, because 24-h urinary excretion of these metabolites not only reflects metabolites of endogenously produced NO but also reflects the daily intake of nitrates. We are confident that the dose of \( \text{L-NAME} \) used in our study produces maximal inhibition of NO synthesis, because we previously showed that higher doses of \( \text{L-NAME} \) do not produce further elevations in arterial pressure or reductions in renal hemodynamics in conscious dogs.

**Perspectives**

Chronic aldosterone excess in normal dogs resulted in a transient sodium retention that was associated with significant increases in arterial pressure, renal plasma flow, and glomerular filtration rate. An important finding of this study is that these changes are accompanied by a significant increase in urinary excretion of nitrate/nitrite, indicating enhanced NO production during chronic aldosterone excess. Another important finding of this study is that although inhibition of NO synthesis does not alter the sodium-retaining or arterial pressure response to aldosterone excess, the renal hemodynamic responses to aldosterone are markedly attenuated in dogs treated with \( \text{L-NAME} \). These results indicate that NO plays an important role in mediating the renal vasodilation during chronic aldosterone excess. Our results also indicate that chronic renal vasodilation and hyperfiltration are not essential for the kidney to escape from the sodium-retaining actions of aldosterone.
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