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Oral administration of L-arginine decreases blood pressure and increases renal excretion of sodium and water in renovascular hypertensive rats

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Abstract

The two-kidney, one-clip renovascular (2K1C) hypertension model is characterized by a reduction in renal flow on the clipped artery that activates the renin-angiotensin system. Endothelium dysfunction, including diminished nitric oxide production, is also believed to play a role in the pathophysiology of this model. Some studies have shown an effect of L-arginine (L-Arg, a nitric oxide precursor) on hypertension. In the present study we determined the ability of L-Arg (7 days of treatment) to reduce blood pressure and alter renal excretions of water, Na^+ and K^+ in a model of 2K1C-induced hypertension. Under ether anesthesia, male Wistar rats (150-170 g) had a silver clip (0.20 mm) placed around the left renal artery to produce the 2K1C renovascular hypertension model. In the experimental group, the drinking water was replaced with an L-Arg solution (10 mg/ml; average intake of 300 mg/day) from the 7th to the 14th day after surgery. Sham-operated rats were used as controls. At the end of the treatment period, mean blood pressure was measured in conscious animals. The animals were then killed and the kidneys were removed and weighed. There was a significant reduction of mean blood pressure in the L-Arg-treated group when compared to control (129 ± 7 *vs* 168 ± 6 mmHg, N = 8-10 per group; $P<0.05$). Concomitantly, a significant enhancement of water and Na^+ excretion was observed in the 2K1C L-Arg-treated group when compared to control (water: 13.0 ± 0.7 *vs* 9.2 ± 0.5 ml/day, $P<0.01$; Na^+: 1.1 ± 0.05 *vs* 0.8 ± 0.05 mEq/day, respectively, $P<0.01$). These results show that orally administered L-Arg acts on the kidney, possibly inducing changes in renal hemodynamics or tubular transport due to an increase in nitric oxide formation.

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Key words
- L-arginine
- Nitric oxide
- Renovascular hypertension
- Sodium excretion
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- Blood pressure

Introduction

There is a controversy in the literature about whether a defect in endothelium-dependent vasodilation contributes through the facilitation of renal vasoconstriction and sodium retention (1,2) to the development of arterial hypertension in some models. The two-kidney, one-clip (2K1C) renovascular hypertension model is characterized by a reduction in renal flow in the left (clipped) renal artery (3). In the chronic phase of 2K1C hypertension the elevation in blood pressure is due mainly to an increase in peripheral vascular resistance, with an additional reduction in venous tone. Despite the associ-

ated reductions in vascular capacity and cardiac output, the glomerular filtration rate in the clipped kidney is reduced and there is an inadequate increase in the glomerular filtration rate of the non-clipped kidney. Thus, it has been suggested that alterations in renal sodium balance may contribute to the hypertension obtained with this model (4).

Nitric oxide (NO) is produced endogenously by a family of NO synthases that utilize L-arginine (L-Arg) as substrate (5). Several studies have demonstrated an important effect of L-Arg on several hypertension models (6-8). It has been postulated that NO contributes to both the early and chronic phases of 2K1C hypertension since its vasodilator effect is able to buffer the hypertension and to maintain the perfusion of both kidneys by counterbalancing angiotensin-independent vasoconstriction (9). NO achieves vasodilation via activation of a soluble guanylate cyclase, which initiates a cascade of events resulting in smooth muscle cell relaxation (10,11). These properties suggest that the level of NO production by the endothelium can play a pivotal role in the regulation of vascular tonus under both physiological and pathological conditions (12,13). Therefore, administration of exogenous L-Arg could, via production of NO, restore endothelial dysfunction and reduce blood pressure in experimental hypertension (14,15).

The role of sodium and water retention has also been studied in the 2K1C model. In dogs, acute changes in renal arterial pressure cause changes in intrarenal NO synthase activity, which may be responsible for the associated changes in sodium excretion (16). Infusion of L-Arg causes profound systemic and renal hemodynamic changes that may be related to changes in water and sodium excretion in the 2K1C hypertension model (9,17,18). Therefore, the present study was carried out to determine if orally administered L-Arg decreases blood pressure in 2K1C hypertensive rats, and its effects on, and correlation with, changes in renal water and sodium excretion.

Material and Methods

Animals

Male normotensive Wistar rats weighing 150-170 g, obtained from the Federal University of Espírito Santo, were used. The animals were housed in a temperature- and humidity-controlled room (25ºC) with a 12-h light cycle. Standard food pellets and tap water were provided *ad libitum*. All experiments were carried out in accordance with the guiding principles for biomedical research involving animals as stated by the Federation of Brazilian Societies of Experimental Biology.

Surgical procedures

Under ether anesthesia a 0.20-mm internal diameter silver clip was placed around the left renal artery through a flank incision. Sham-operated rats underwent a similar procedure with manipulation of the left renal artery but without permanent application of the clip. The experimental groups (2K1C and sham-operated) were given L-Arg (Sigma, St. Louis, MO, USA) dissolved in the drinking water (10 mg/ml), while the respective control groups (N = 8-10 per group) received only water. The daily intake of L-Arg was estimated to be 300 mg.

Experimental protocol

Seven days after surgery (2K1C or sham), the L-Arg treatment was initiated and the rats were housed individually in metabolic cages for 24-h urine collection for urinary sodium and potassium assay by flame photometry (Micronal B262, São Paulo, SP, Brazil). Urine volume and water intake volume were determined daily during treatment. Systolic blood pressure was measured by tail-cuff plethysmography on days 7 and 14 after surgery. On the morning of day 15, animals were anesthetized with ether and a catheter

made of PE-50 tubing connected to PE-10 tubing was passed through the right femoral artery. The catheter was tunneled to exit at the back of the neck, flushed and filled with 40 U/ml heparinized saline. About 9 h later, mean arterial pressure was measured in conscious, freely moving animals using a pressure transducer (model PT 300; Grass Instruments Div., Warwick, NY, USA) coupled to a Biopac System (MP100, Santa Barbara, CA, USA). At the end of each experiment, the left and right kidneys were removed and decapsulated and the wet and dry kidney weight/body weight ratios were determined.

Statistical analysis

Data for mean arterial pressure, left kidney/body weight and right kidney/body weight ratios, water intake, sodium excretion and potassium excretion were analyzed by one-way ANOVA for repeated measures, followed by the Tukey test for comparison of the means. Differences were considered significant when $P<0.05$.

Results

Table 1 shows indirect arterial blood pressures obtained on the 7th and 14th day after surgery in all experimental groups (2K1C and sham groups, treated or not with L-Arg). The evolution of hypertension amongst the groups was different. In the sham-operated group, L-Arg administration did not modify the basal systolic blood pressure when compared with the respective untreated group by 7 (110 ± 4 and 110 ± 4 mmHg) and 14 days after surgery (110 ± 5 and 109 ± 5 mmHg, respectively). In the resulting hypertensive groups, L-Arg treatment evoked a different (less intense) development of hypertension, as shown by indirect tail-cuff measurements. In these hypertensive groups, the values of systolic blood pressure were similar before treatment with L-Arg (170 ± 7 and 173 ± 7 mmHg); however, on day 14 of hypertension and 7 days after the beginning of L-Arg treatment, the 2K1C L-Arg-treated animals showed a significant reduction in blood pressure levels when compared with untreated 2K1C rats (143 ± 5 *vs* 183 ± 8 mmHg; $P<0.05$).

These results were confirmed by direct measurement of mean blood pressure. As illustrated in Figure 1 and summarized in Figure 2, L-Arg treatment produced a significant decrease in hypertension in 2K1C rats when compared with untreated 2K1C animals (129 ± 7 *vs* 168 ± 6 mmHg, respectively; $P<0.01$). This antihypertensive effect

Table 1. Effects of L-arginine (L-Arg) administration on basal systolic blood pressure (SBP; indirect measure) before (7 days after two-kidney, one-clip renovascular (2K1C) surgery) and 14 days after 2K1C surgery of normotensive sham-operated and 2K1C hypertensive rats.

Group	SBP 7th day (mmHg)	SBP 14th day (mmHg)
Sham	110 ± 4 (8)	109 ± 5 (8)
Sham L-Arg	110 ± 4 (8)	110 ± 5 (8)
2K1C	173 ± 7 (10)*	183 ± 8 (10)*
2K1C L-Arg	170 ± 7 (10)$^{+}$	143 ± 5 (10)$^{+\#}$

Rats received L-Arg (10 mg/ml) in drinking water. The number of rats in each group is given in parentheses. Data are reported as means ± SEM. *$P<0.01$ compared with sham; $^{+}P<0.01$ compared with sham L-Arg; $^{\#}P<0.01$ compared with 2K1C rats (Tukey test).

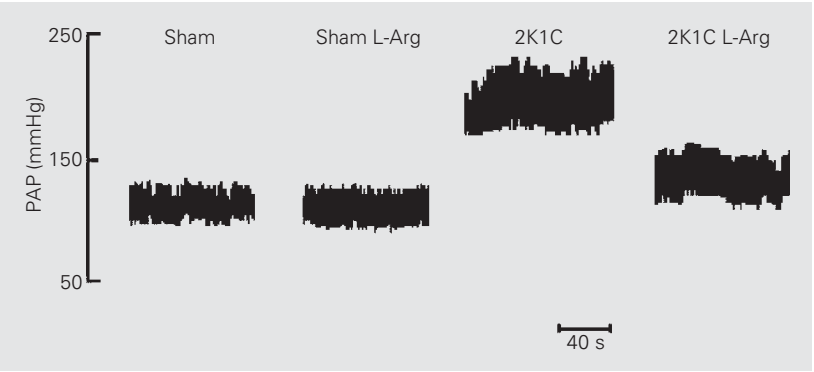

Figure 1. Typical recording of pulsatile arterial pressure (PAP) for the four experimental groups: sham (sham-operated), sham L-Arg (sham treated with L-arginine), two-kidney, one-clip (2K1C) control and 2K1C L-Arg-treated rats. The data are shown for 14 days of L-Arg administration.

of L-Arg treatment occurred simultaneously with the increase in water and sodium excretion in 2K1C-treated rats (Figures 3 and 4). The daily mean fluid intake and excretion were higher in 2K1C L-Arg-treated rats (39 ± 1.1 and 13 ± 0.7 ml/day, respectively) compared to untreated 2K1C hypertensive rats (32 ± 0.9 and 9.2 ± 0.5 ml/day). The sham group treated with L-Arg also exhibited an increase in water intake and excretion (38 ± 0.9 and 11 ± 0.6 ml/day) compared to the untreated sham group (28 ± 0.8 and 7.6 ± 0.2 ml/day).

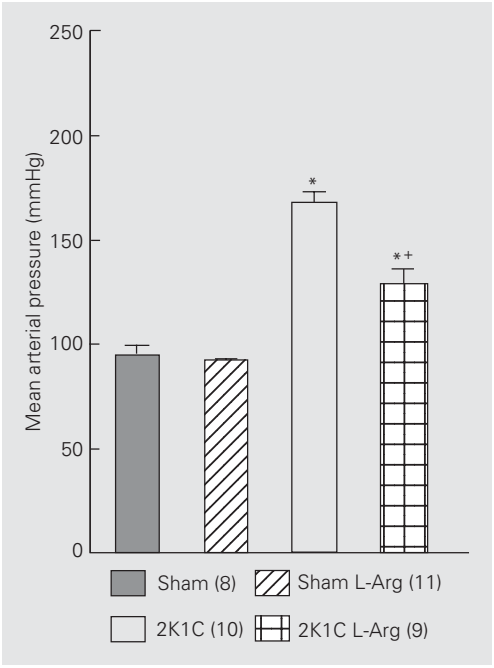

Figure 2. Effect of oral L-arginine (L-Arg) administration on mean arterial pressure (direct measurement) of sham, sham L-Arg, two-kidney, one-clip (2K1C) and 2K1C L-Arg groups. The data are shown for 14 days of L-Arg administration. Data are reported as means ± SEM. The number of animals in each group is given in parentheses. *P<0.01 compared with sham; +P<0.01 compared with 2K1C (Tukey test).

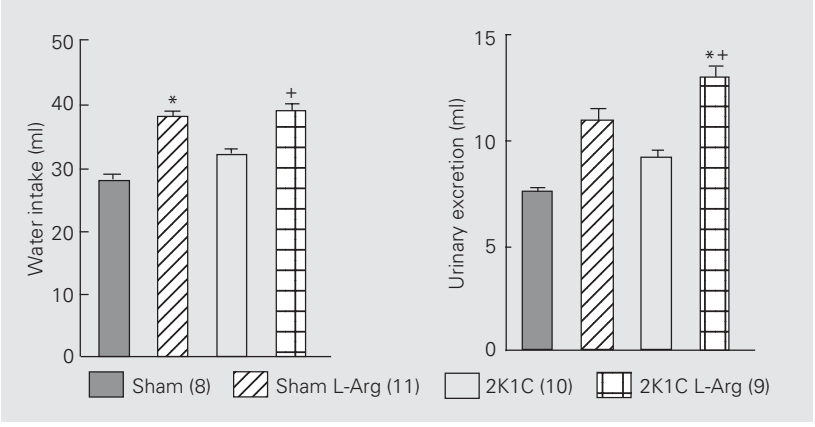

Figure 3. Water intake and excretion after oral L-arginine (L-Arg) administration to the sham and two-kidney, one-clip (2K1C) groups. The data are shown for 7 days of L-Arg administration. Data are reported as means ± SEM. The number of animals in each group is given in parentheses. *P<0.01 compared with sham; +P<0.01 compared with 2K1C (Tukey test).

Sodium excretion was also higher in 2K1C rats treated with L-Arg compared to the untreated 2K1C group (1.1 ± 0.05 and 0.8 ± 0.05 mEq/day, respectively; Figure 4). An increase in sodium excretion was already observed 24 h after the oral administration of L-Arg, and remained high throughout the 7-day collection period (data not shown).

Sodium excretion by the sham groups was similar in L-Arg-treated (0.9 ± 0.07 mEq/day) and untreated (0.7 ± 0.02 mEq/day) rats. Potassium excretion was similar in all groups: sham water (1.4 ± 0.04), sham L-Arg (1.5 ± 0.1), 2K1C water (1.5 ± 0.07) and 2K1C L-Arg (1.8 ± 0.08 mEq/day).

Baseline dry kidney/body weight ratios of the rats are shown in Table 2. This renovascular hypertension model is associated with a significant decrease in the weight of the clipped (left) kidney. L-Arg treatment increased the wet and dry weights of the clipped kidney (3.0 ± 0.2; 0.63 ± 0.05 mg/g) when compared with the untreated 2K1C group (2.0 ± 0.2; 0.45 ± 0.03 mg/g). The non-clipped kidneys (right) did not differ in weight between the treated (4.5 ± 0.2; 1.0 ± 0.03 mg/g) and untreated hypertensive groups (4.3 ± 0.1; 1.0 ± 0.11 mg/g).

Discussion

The main finding of the present study was that L-Arg orally administered for 7 days significantly reduced blood pressure by 23% in rats with 2K1C-induced hypertension. It has been shown that L-Arg does not decrease blood pressure in renovascular hypertensive dogs (19), a fact probably due to a much lower dose (75 mg/day) of the NO precursor used and/or to species differences. Our results with regard to the increase of water and sodium excretion also suggest that the antihypertensive effect of L-Arg could

be partially due to the diuretic and natriuretic effect of the NO precursor.

In chronic 2K1C hypertension, in addition to the influence of the renin-angiotensin system on peripheral resistance, abnormalities in renal sodium handling have been linked to the pathogenesis of hypertension (5). On the other hand, alterations in renal hemodynamics by intra- or extrarenal angiotensin II can reduce the ability of the kidney to excrete sodium (20,21). It has been established that in the 2K1C model glomerular filtration rate is reduced in the clipped kidney, and alterations in renal handling of sodium contribute to the hypertension (5). Mohring and colleagues (22) have demonstrated a significant correlation between increased blood pressure and sodium retention in rats with 2K1C hypertension, suggesting that a positive sodium balance plays an important role in the pathogenesis of this renovascular hypertension (23). These data are consistent with the present observations concerning the natriuresis that follows the administration of L-Arg to 2K1C rats.

It has been proposed that various forms of hypertension are associated with a dysfunctional endothelium due to the deficient production of endothelium-derived NO (10). Observations in isolated vessels in a number of experimental models of hypertension, including 2K1C renovascular hypertensive rats, have suggested that endothelium-dependent vasodilatation is reduced in these models (1,2,10,15). It seems that endogenously produced NO protects the kidney against the effect of angiotensin II on the glomerular filtration system and on renal flow (15,24). On the other hand, infusion of L-Arg (as a precursor of NO) produces vasodilation and changes in renal hemodynamics in human hypertension (25). L-Arg infusion also causes renal vasodilatation and natriuresis (26-28). Although we have not tested D-Arg in the present study, it was recently shown that L-Arg, but not D-Arg, prevents the hypertension in Dahl salt-sensitive rats (29).

The controversy about the levels of NO production in the 2K1C model should be mentioned. It has been suggested that NO activity is higher than normal in the non-clipped kidney, and there is evidence that up-regulation of endothelium NO synthase occurs in this model (30). One possible interpretation of our data is that here we refer to systemic formation of NO, while the former investigators refer to local production of NO in an acute situation (in contrast to the 15-day period of our study). In support of our view, there is evidence that renovascular

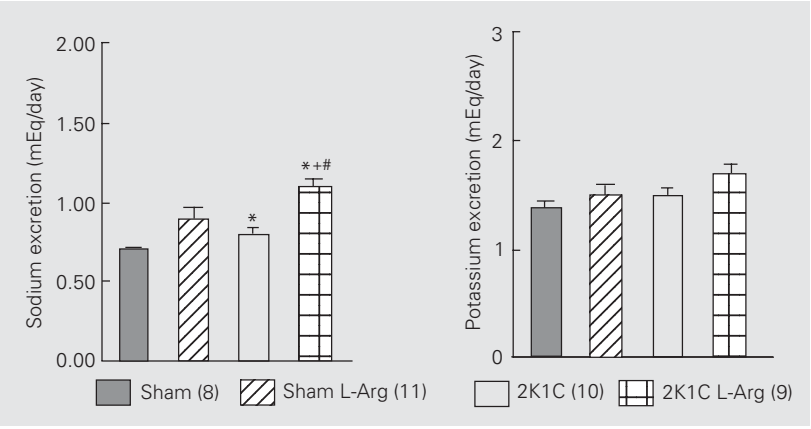

Figure 4. Urinary sodium and potassium excretion of sham-operated and two-kidney, one-clip (2K1C) rats treated or not with oral L-arginine (L-Arg). The data are shown for 7 days of L-Arg administration. Data are reported as means ± SEM. The number of animals in each group is given in parentheses. *P<0.01 compared with sham; $^{+}$P<0.01 compared with sham L-Arg; $^{\#}$P<0.01 compared with 2K1C (Tukey test).

Table 2. Wet and dry kidney/body weight ratios of clipped (left) and non-clipped (right) kidneys of sham and two-kidney, one-clip (2K1C) renovascular hypertensive rats treated or not with L-arginine (L-Arg).

Group	Wet kidney/body weight ratio (mg/g)		Dry kidney/body weight ratio (mg/g)	
	Left	Right	Left	Right
Sham	2.9 ± 0.08	2.9 ± 0.08	0.78 ± 0.02	0.76 ± 0.01
Sham L-Arg	2.9 ± 0.08	3.0 ± 0.16	ND	ND
2K1C	2.0 ± 0.2*$^{\#}$	4.3 ± 0.1*	0.45 ± 0.03*$^{\#}$	1.0 ± 0.11*
2K1C L-Arg	3.0 ± 0.2	4.5 ± 0.2‡	0.63 ± 0.05^{+}	1.0 ± 0.03

Rats received L-Arg (10 mg/ml) in drinking water. The number of rats in each group is the same as in Table 1. Data are reported as means ± SEM. *P<0.01 compared with sham; $^{+}$P<0.05 compared with 2K1C; ‡P<0.01 compared with sham L-Arg; $^{\#}$P<0.01 compared with 2K1C L-Arg (Tukey test). ND, not determined.

hypertension is associated with an increase in oxidative stress which could reduce the bioavailability of NO and impair endothelium-dependent vasodilatation (31). Our results, taken together with literature data (17,25-28), indicate that renal production of NO is important in the regulation of sodium and water excretion.

These data support the hypothesis that in renovascular hypertension L-Arg treatment decreases the arterial pressure not only because of the already known vasodilator effects of NO formation, but also because there is an increase in renal excretion of water and sodium. These diuretic and natriuretic effects of L-Arg may result from direct alterations in renal hemodynamics and/or by opposing the effects of the renal renin-angiotensin system on the water and sodium balance.

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