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Participation of kinins in the inhibitory action of captopril on acute hypertension induced by L-NAME in anesthetized rats

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Abstract

The aim of the present study was to investigate the role of bradykinin in the inhibitory action of captopril in hypertension induced by L-NAME in anesthetized rats. Male Wistar rats (260-320 g) were anesthetized with chloralose and arterial blood pressure was recorded with a polygraph pressure transducer. The hypertensive effect of L-NAME was studied in rats pretreated with saline, captopril or HOE 140 plus captopril. The effect of captopril was also studied during the sustained pressor effect of L-NAME. The acute pressor effect of L-NAME (10 mg/kg, *iv*) was significantly reduced by *iv* pretreatment with 2 mg/kg captopril (Δ increase of 49 ± 4.9 mmHg reduced to 20 ± 5.4 mmHg, P = 0.01). The pressor effect of L-NAME (Δ increase of 38 ± 4.8 mmHg) observed in rats pretreated with captopril and HOE 140 (0.1 mg/kg, *iv*) was not significantly different from that induced by L-NAME in rats pretreated with saline (P = 0.09). During the sustained pressor effect induced by L-NAME (Δ increase of 49 ± 4.9 mmHg) captopril induced a significant (P<0.05) reduction in arterial blood pressure (Δ decrease of 22 ± 3.0 mmHg). The present results demonstrate that the acute pressor effect of L-NAME is reduced by captopril and this inhibitory effect may be partly dependent on the potentiation of the vasodilator actions of bradykinin.

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Inhibition of nitric oxide (NO) synthesis by L-NAME induces acute (1) and chronic (2) increases in arterial blood pressure in experimental animals, thus providing a useful model for the study of the pathophysiology of arterial hypertension. How the lack of NO synthesis induces arterial hypertension is open to discussion. Anti-hypertensive compounds can be used as tools to study the mechanisms that participate in the development of arterial hypertension during chronic systemic L-NAME administration. Chronic arterial hypertension induced by L-NAME treatment is significantly prevented by simultaneous treatment with losartan (2), captopril (3) or ramipril (4). Captopril also reverses the chronic increase in arterial blood pressure induced by L-NAME (3). These experimental findings suggest an important role for the renin-angiotensin system in the genesis of L-NAME-induced arterial hypertension. However, since captopril is an inhibitor of kininase II (EC 3.4.15.1), the enzyme that forms angiotensin II and also inac-

tivates bradykinin, it is possible that bradykinin, a potent releaser of endothelium-derived relaxant factor (5), may also play a significant role in the anti-hypertensive effect of captopril on hypertension induced by L-NAME. The present study was designed to investigate the role of endogenous kinins in the inhibitory action of captopril on the pressor effect of L-NAME. For this purpose, HOE 140, a specific and long-lasting bradykinin B2 receptor antagonist (6), was used.

Experiments were performed on male Wistar rats (260-320 g) bred in the animal house of the Department of Pharmacology, UERJ. Under ether anesthesia, a polyethylene catheter was implanted into the femoral vein of the animals. After discontinuation of ether, the rats were kept anesthetized with chloralose (100 mg/kg, *iv*). Another catheter was implanted into the femoral artery to record mean arterial blood pressure with a polygraph (Nihon Koden). Thirty minutes after the beginning of arterial blood pressure recording (equilibration period), four experimental protocols were performed.

Protocol 1. Thirty minutes after injection of 0.2 ml saline, L-NAME (10 mg/kg) was injected into the femoral vein followed by intravenous injection of captopril (2 mg/kg) 30 min later. *Protocol 2*. Captopril (2 mg/kg) was injected into the femoral vein followed 15 min later by intravenous injection of HOE 140 (0.1 mg/kg) and 30 min later by intravenous injection of L-NAME (10 mg/kg). *Protocol 3*. Captopril (2 mg/kg) was injected into the femoral vein followed 15 min later by intravenous injection of HOE 140 (0.1 mg/kg) and 30 min later by intravenous injection of L-NAME (10 mg/kg). *Protocol 4*. Bradykinin (100 ng/kg) was injected into the femoral vein before and 30 min after intravenous injection of HOE 140 (0.1 mg/kg).

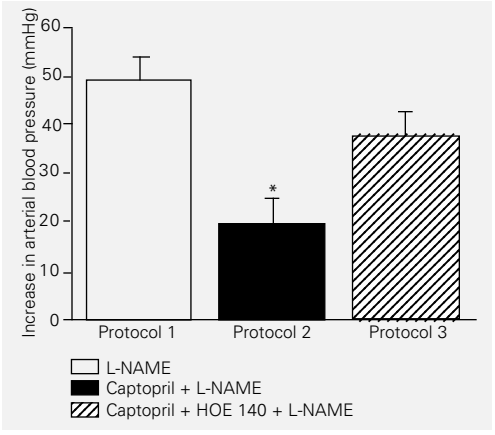

Figure 1 - Increase in mean arterial blood pressure (mmHg) induced by L-NAME according to protocols 1, 2 and 3 as described in text. Bars indicate the mean ± SEM. *P<0.05 *vs* protocol 1 (Student *t*-test).

Captopril, L-NAME, bradykinin and chloralose were purchased from Sigma Chemical Co., St. Louis, MO. HOE 140 was a gift from Hoechst AG, Frankfurt, Germany. Captopril, bradykinin and L-NAME were dissolved in distilled water on the day of the experiment. Stock solutions of HOE 140 (1 mg/ml) in distilled water were stored frozen at -20ºC and dissolved in distilled water on the day of the experiment.

Results are reported as mean ± SEM; N represents the number of rats in each group. The data were analyzed statistically using the Student *t*-test for unpaired observations, with the level of significance set at $P<0.05$.

Intravenous injection of L-NAME followed by intravenous injection of captopril (protocol 1). Mean arterial blood pressure before L-NAME injection was 97 ± 3 mmHg (N = 9). Intravenous injection of L-NAME produced a slow increase in arterial blood pressure that reached its maximal level (49 ± 4.9 mmHg; Figure 1) after 15 min and was sustained for more than 30 min. Intravenous injection of captopril performed 30 min after L-NAME administration produced a hypotensive response of 22 ± 3.0 mmHg (Table 1).

Intravenous injection of L-NAME after intravenous injection of captopril (protocol 2). Mean arterial blood pressure before captopril injection was 107 ± 4 mmHg (N = 9). Intravenous injection of captopril produced a sustained hypotensive effect of 14 ± 4 mmHg; 30 min after captopril injection, arterial blood pressure was 93 ± 6 mmHg. At this moment, intravenous injection of L-NAME produced a sustained pressor response of 20 ± 5 mmHg. This pressor response was significantly different (P = 0.01) from the pressor response obtained in proto-

Table 1 - Effect of captopril and HOE 140 on L-NAME-induced hypertension.

Mean arterial blood pressure is reported in mmHg. Results are reported as the mean ± SEM for 9 rats in each group. Pressure measurements are reported 15 or 30 min after *iv* administration of each substance. *P<0.05 compared to protocol 1 (Student *t*-test).

Protocol 1 (N = 9)			
Control	30 min after L-NAME	15 min after captopril	
97 ± 3	146 ± 5	124 ± 5	
Protocol 2 (N = 9)			
Control	30 min after captopril	15 min after L-NAME	
107 ± 4	93 ± 6	113 ± 8*	
Protocol 3 (N = 9)			
Control	15 min after captopril	15 min after HOE 140	15 min after L-NAME
102 ± 3	85 ± 6	88 ± 7	126 ± 6

col 1 (Figure 1 and Table 1).

Intravenous injection of L-NAME after intravenous injection of captopril and HOE 140 (protocol 3). Mean arterial blood pressure before captopril injection was 102 ± 3 mmHg (N = 9). Intravenous injection of captopril produced a sustained hypotensive effect of 17 ± 3 mmHg; 15 min after captopril injection, arterial blood pressure was 85 ± 6 mmHg and intravenous injection of 0.1 mg/kg HOE 140 performed at this moment produced no significant change in arterial blood pressure. Fifteen minutes after HOE 140 injection, i.e., 30 min after captopril injection, arterial blood pressure was 88 ± 7 mmHg. At this moment, intravenous injection of L-NAME produced a sustained response of 38 ± 4.8 mmHg similar (P = 0.09) to the pressor response obtained in protocol 1 (Figure 1 and Table 1).

Intravenous effect of bradykinin before and after HOE 140 (protocol 4). Mean arterial blood pressure observed before bradykinin injection was 109 ± 3 mmHg (N = 5). Intravenous injection of 100 ng/kg bradykinin induced a rapid decrease in arterial blood pressure of 40 ± 6.0 mmHg. Intravenous injection of 0.1 mg/kg HOE 140 did not change mean arterial blood pressure. Intravenous injection of bradykinin (100 ng/kg) 15 min after HOE 140 induced no change in arterial blood pressure.

The present results confirm the previous observation that intravenous injections of L-NAME into anesthetized rats induce an acute and sustained increase in arterial blood pressure. This increase in arterial blood pressure is probably independent of cardiac stimulation since intravenous injection of L-NAME induces bradycardia and a fall in cardiac output in rats (7). An increase in arterial vascular resistance due to general vasoconstriction seems to play the most important role in the genesis of the pressor response induced by inhibitors of NO synthesis (7). The mechanisms increasing vascular smooth muscle contractility have not been completely established. The hypertensive effects of inhibitors of NO synthase may be caused by suppression of the basal vasodilator effect of NO (8), by stimulation of central sympathetic activity (9), by unmasking of an endothelium-induced vasopressor response (10) and by enhancement of the pressor activity of autacoids or neurotransmitters involved in the control of arterial blood pressure (11).

The effect of kininase II inhibitors on the acute pressor effect induced by nitric oxide blockade is controversial. The acute pressor effect induced by inhibition of nitric oxide synthesis was not changed by pretreatment of rats with captopril (11,12) or enalapril (13). However, in dogs the hypertensive effect of L-NAME is reduced by captopril

(14). Our results show that in anesthetized rats captopril significantly reduces the L-NAME-sustained pressor response. The present results are in accordance with our previous findings obtained in rats treated chronically with L-NAME and captopril (3). However, while in unanesthetized rats chronic captopril treatment completely prevented the increase in arterial blood pressure and also normalized the hypertension induced by L-NAME (3), our present results of acute treatment indicate that in anesthetized rats this phenomenon seems to occur in a different way. Intravenous injections of captopril during the pressor effect of L-NAME significantly reduced but did not abolish the rise in arterial blood pressure induced by L-NAME. Furthermore, the pressor effect of L-NAME was reduced but not completely abolished by captopril pretreatment. This difference may be due to an insufficient dose of captopril or to the actions of kininase II inhibitors that only appear during chronic treatment (15).

The mechanism by which captopril reduces the increase in arterial blood pressure induced by L-NAME has not yet been clarified. The effect of captopril may be due to inhibition of kininase II, that normally converts angiotensin I to angiotensin II, since potentiation of the pressor effect of angiotensin II (11) has been demonstrated in unanesthetized rats under NO inhibition. The effect of captopril may be also modulated by reduction of the pressor effect of vasopressin, an autacoid released by the action of angiotensin II on the CNS, since the pressor effect of intravenous infusion of L-NAME in dogs is significantly inhibited by vasopressin-V1 blockade (16). Captopril may also interfere directly with autacoids released by the endothelium since this compound induces endothelium-dependent relaxation in isolated rabbit aortic rings precontracted with norepinephrine (17).

Vascular actions of bradykinin, a substance that induces release of vasodilator substances by the endothelium (5), may also play an important role in the inhibitory effect of captopril. We have shown that this inhibitory effect on the pressor response of L-NAME is significantly reduced by pretreatment with HOE 140, a compound that selectively antagonizes bradykinin B2 receptors. Therefore, these results suggest that this inhibitory effect is dependent on potentiation of the hypotensive effect of bradykinin. These data are in accordance with previous findings describing the effect of bradykinin antagonism on the hypotensive and anti-hypertensive actions of kininase II inhibitors (18-20). Potentiation of the bradykinin effects by captopril (5) may be a mechanism that, by interacting with the inhibition of angiotensin II formation, may explain part of the inhibitory effect of captopril observed in the present study. In conclusion, the present results suggest that captopril reduces the acute increase in arterial blood pressure in anesthetized rats and also decreases the sustained high levels of arterial blood pressure induced by L-NAME. Potentiation of the bradykinin effects probably plays an important role in this inhibitory effect of captopril on the pressor effect of L-NAME.

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