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European Journal of Pharmacology 342 (1998) 285–289

ejp

Short communication

A nitric oxide donor NOC 7 suppresses renal responses induced by norepinephrine and angiotensin II in the NO-depleted denevated rabbit kidney

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Received 28 November 1997; accepted 2 December 1997

Abstract

Intrarenal arterial infusion of norepinephrine (30 ng/kg per min) or of angiotensin II (4 ng/kg per min) reduced the glomerular filtration rate and urinary Na^+ excretion in denervated kidneys of anesthetized rabbits pretreated intrarenally with a nitric oxide (NO) synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (50 μg/kg per min). Angiotensin II but not norepinephrine reduced fractional Na^+ excretion. Intrarenal administration of a spontaneous NO donor 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC 7, 30 ng/kg per min) in L-NAME pretreated kidneys did not affect basal values, but attenuated the reduction in urinary Na^+ excretion induced by these agonists without affecting the angiotensin II-induced reduction in glomerular filtration rate. The results suggest that NOC 7 can suppress the norepinephrine-induced hypofiltration and the angiotensin II-evoked tubular reabsorption and thereby attenuates the agonist-induced antinatriuresis in the denervated and endogenous NO-depleted rabbit kidney. © 1998 Elsevier Science B.V.

Keywords: Norepinephrine; Angiotensin II; Nitric oxide (NO); N^{ω}-nitro-L-arginine methyl ester (L-NAME); NOC 7 (1-hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-1-triazene); Kidney; (Rabbit)

1. Introduction

Nitric oxide (NO) has been suggested to contribute to maintaining renal circulation and urine formation and modulate neural and humoral control of the renal functions. NO synthase inhibitors such as *N*-monomethyl-L-arginine, N^G-nitro-L-arginine or N^{ω}-nitro-L-arginine methyl ester (L-NAME) induce renal vasoconstriction and antinatriuresis (Baylis et al., 1990; Tolins et al., 1990; Majid et al., 1993) and facilitate norepinephrine- and angiotensin II-induced renal vasoconstriction and antinatriuresis (Ohishi et al., 1992; Matsumura et al., 1995; Adachi et al., 1996) in experimental animals. Parakh et al. (1996) reported that an authentic NO donor sodium nitroprusside attenuated a reduction in renal blood flow induced by intrarenal arterial infusion of norepinephrine and angiotensin II in the absence or presence of L-NAME in anesthetized rats, confirming the counteracting effects of NO on the vasoconstrictor stimuli in vivo. However, it was unclear whether the elevation of NO level induced by NO donors affects the agonist-induced changes in urine formation.

We have recently demonstrated that a novel NO donor 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC 7; Hrabie et al., 1993) attenuated norepinephrine- and angiotensin II-induced antinatriuresis which may be due mainly to enhanced tubular Na^+ reabsorption and reduced glomerular filtration, respectively, in the absence of L-NAME in anesthetized rabbits (Adachi et al., 1997). Unlike sodium nitroprusside, NOC 7 spontaneously releases NO in biological fluid and dose not produce toxic products such as cyanide (Hrabie et al., 1993) and is considered to be useful as a short-acting nitrovasodilator that has no major adverse effect or tolerance (Zhang et al., 1996).

Our previous study, however, suggested that the angiotensin II-induced antinatriuresis involves enhanced tubular Na^+ reabsorption in the presence of L-NAME (Adachi et al., 1996). Thus, endogenously formed NO may

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alter contribution of the renal filtration and reabsorption components to agonist-induced antinatriuretic responses.

In the present study, to clarify further the counteracting effect of NO on the agonist-induced antinatriuresis, we examined whether NOC 7 can suppress the norepinephrine- and angiotensin II-induced renal responses in the presence of L-NAME in anesthetized rabbits.

2. Materials and methods

2.1. Preparation

Male Japanese white rabbits (2.5–3.5 kg) were anesthetized with sodium pentobarbital (40 mg/kg) injected through a marginal ear vein. The trachea was cannulated and the rabbit was artificially ventilated with room air (stroke volume 50 ml, 25 rpm). A double lumen catheter was inserted into the right femoral vein for drug administration. Anesthesia was maintained by continuous infusion of pentobarbital (2–4 mg/kg per h, i.v.) throughout the experiments. Inulin, dissolved in plasma extender solution (consisting of NaCl, 0.5 g; KCl, 0.03 g; $CaCl_2$, 0.02 g; glucose, 1.5 g; sodium lactate, 0.224 g in 100 ml), was given i.v. at a priming dose of 50 mg/kg and at a maintenance dose of 1 mg/kg per min (0.1 ml/kg per min). The right femoral artery was cannulated for collection of arterial blood samples and measurement of arterial blood pressure with a pressure transducer (model TP-200T, Nihon Kohden, Tokyo, Japan). The left kidney was exposed by retroperitoneal flank incision and the animal was suspended by clamping one of its lumbar spinous processes to facilitate manipulation. A catheter for urine collection was inserted into the ureter. All visible renal nerves were dissected away from the renal vessels and cut after ligation to exclude possible effects of drugs on the neural control of renal function. An electromagnetic flow probe (1.5 mm in diameter, Nihon Kohden) was attached to the renal artery to measure renal blood flow with a square-wave flowmeter (model MF-27, Nihon Kohden). A curved 30-gauge needle connected to PE 10 tubing with four side branches (for single or combined intrarenal arterial infusion of drugs) was placed into the renal artery for drug infusion. Heparinized 0.9% saline (250 units/ml) was continuously infused via this catheter (1.0 ml/h) throughout the experiments. Blood pressure, heart rate and renal blood flow were recorded with a polygraph system (model PM-6000, Nihon Kohden). After completion of surgery, more than 90 min were allowed for stabilization with continuous monitoring of urine flow and hemodynamics. The animals were divided into four groups.

2.2. Experimental protocols

2.2.1. Group 1 (n = 8)

Urine was collected over a 10-min period and 0.6 ml of arterial blood was withdrawn at the midpoint of urine collection to obtain basal values. Norepinephrine was then infused into the renal artery at 30 ng/kg per min for 15 min. 5 min after the start of norepinephrine infusion, 10-min urine sampling and blood sampling were performed and the infusion was stopped. 10 min after the end of norepinephrine infusion, urine and blood samples for recovery values were collected. Then intrarenal arterial infusion of L-NAME at 50 μg/kg per min was started. 30 min after the start of the infusion, urine and blood sampling and norepinephrine infusion were performed again.

2.2.2. Group 2 (n = 8) and group 3 (n = 8)

L-NAME (50 μg/kg per min) was infused into the renal artery throughout the experiments. 30 min after the start of L-NAME infusion, urine and blood sampling and intrarenal arterial infusion of norepinephrine (30 ng/kg per min, group 2) or of angiotensin II (4 ng/kg per min, group 3) were performed as in group 1. After sampling for recovery values, intrarenal arterial infusion of NOC 7 at 30 ng/kg per min was started. 30 min after the start of NOC 7 infusion, urine and blood sampling and the agonist infusion were performed.

2.3. Measurements

Blood samples were transferred to ice-chilled tubes containing ammonium EDTA (6 mg/ml blood) and then centrifuged to obtain plasma samples. Glomerular filtration rate was determined as inulin clearance. Inulin concentration in plasma and urine was measured by the anthrone method. Na^+ and K^+ were measured by flame photometry (model 775A, Hitachi).

2.4. Statistics

All values were expressed as means ± S.E. The effects of drugs (L-NAME and NOC 7) on the values before and during norepinephrine or angiotensin II infusion and during the recovery period were analyzed by analysis of variance (ANOVA) for multifactor repeated measures. When ANOVA showed a statistical difference, the values in the control period and in the drug infusion period were compared in terms of simple main effects. Student's paired *t*-test was used to compare values before and during the agonists infusion and the percentage changes induced by the agonists in the control period and the drug infusion period. Differences at $P < 0.05$ were considered to be statistically significant.

3. Results

In the control period of non-pretreated rabbits (group 1), intrarenal arterial infusion of norepinephrine reduced

renal blood flow, urine flow rate, urinary Na^+ excretion and fractional Na^+ excretion (Table 1). After the norepinephrine infusion was stopped, each value returned nearly to its basal level (data not shown). Intrarenal arterial infusion of L-NAME reduced renal blood flow, urine flow rate, urinary Na^+ excretion and fractional Na^+ excretion with little change in glomerular filtration rate (group 1, Table 1). In the L-NAME infusion period, norepinephrine significantly reduced glomerular filtration rate and filtration fraction (group 1, Table 1).

L-NAME infusion potentiated the norepinephrine-induced changes in renal blood flow, urine flow rate, urinary Na^+ excretion (the percent changes from basal level were $-27 \pm 5\%$ in the control period versus $-61 \pm 10\%$ in the L-NAME infusion period) and glomerular filtration rate ($-9 \pm 6\%$ versus $-67 \pm 7\%$) but abolished the change in fractional Na^+ excretion ($-20 \pm 8\%$ versus $14 \pm 18\%$) (group 1).

In L-NAME-pretreated rabbits (group 2), intrarenal arterial infusion of NOC 7 did not affect basal values of hemodynamics or urinary excretion. For example, the basal values in the control and the NOC 7 infusion periods in group 2 were as follows: renal blood flow, 21 ± 2 and 23 ± 2 ml/min; glomerular filtration rate, 3.9 ± 0.3 and 4.3 ± 0.5 ml/min; filtration fraction, 34 ± 2 and $34 \pm 3\%$; urine flow rate, 0.14 ± 0.04 and 0.13 ± 0.03 ml/min; urinary Na^+ excretion, 11.6 ± 4.5 and 13.6 ± 3.6 μEq/min; fractional Na^+ excretion, 2.4 ± 1.0 and $2.3 \pm 0.6\%$, respectively.

Fig. 1 shows the agonists-induced responses (percent changes in the renal hemodynamic and urinary parameters from the basal levels) in groups 2 and 3. NOC 7 attenuated the norepinephrine-induced changes in renal blood flow, urine flow rate, urinary Na^+ excretion ($-48 \pm 7\%$ in the L-NAME infusion period versus $-8 \pm 12\%$ in the L-NAME plus NOC 7 infusion period), glomerular filtration rate ($-40 \pm 10\%$ versus $-2 \pm 12\%$) and filtration fraction (group 2, Fig. 1A).

Angiotensin II reduced renal blood flow, glomerular filtration rate, urine flow rate, urinary Na^+ excretion and fractional Na^+ excretion in L-NAME-pretreated rabbits (group 3, Fig. 1B). NOC 7 attenuated the angiotensin II-induced changes in urine flow rate, urinary Na^+ excretion ($-40 \pm 6\%$ in the L-NAME infusion period versus $-8 \pm 6\%$ in the L-NAME plus NOC 7 infusion period) and fractional Na^+ excretion ($-16 \pm 4\%$ versus $20 \pm 9\%$) but not the change in renal blood flow, glomerular filtration rate or filtration fraction (Fig. 1B).

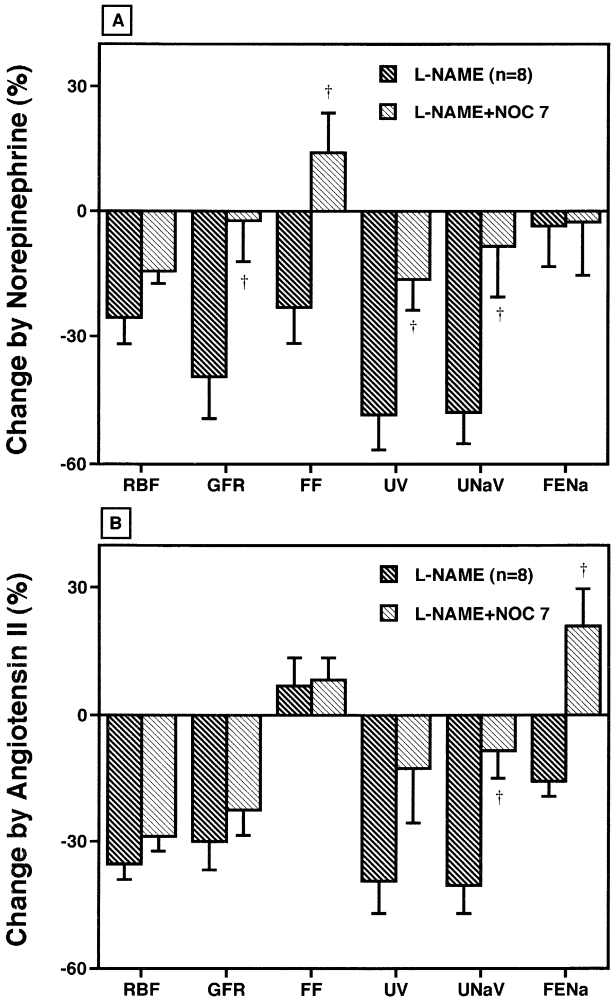

Fig. 1. Effects of NOC 7 in the presence of L-NAME on changes in renal functions induced by norepinephrine (panel A: group 2, $n = 8$) and by angiotensin II (panel B: group 3, $n = 8$). RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate; UNaV, urinary Na^+ excretion; FENa, fractional excretion of Na^+. Values (means ± S.E.) are percent changes from basal values in response to norepinephrine or angiotensin II infusion. L-NAME (50 μg/kg per min) and NOC 7 (30 ng/kg per min) was infused into the renal artery. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with the corresponding control value.

Table 1
Systemic and left kidney hemodynamics and urinary parameters in group 1 ($n = 8$)

	Control		L-NAME	
	basal	NE	basal	NE
MAP (mm Hg)	84 ± 2	86 ± 3	89 ± 3[c]	92 ± 4[b,c]
RBF (ml/min)	22 ± 2	19 ± 2[a]	18 ± 2[d]	12 ± 2[a,c]
GFR (ml/min)	3.1 ± 0.5	2.8 ± 0.4	3.0 ± 0.5	1.1 ± 0.3[a,c]
FF (%)	26 ± 4	27 ± 4	36 ± 9[d]	22 ± 8[b]
UV (ml/min)	0.29 ± 0.06	0.24 ± 0.05[b]	0.16 ± 0.06[d]	0.05 ± 0.02[b,c]
UNaV (μEq/min)	17.6 ± 5.6	13.1 ± 4.2[b]	9.0 ± 4.0[d]	2.5 ± 1.1[d]

Values (means ± S.E.) were obtained before and during norepinephrine infusion: shown as 'basal' and 'NE', respectively. MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate; UNaV, urinary Na^+ excretion; FENa, fractional Na^+ excretion. Norepinephrine (NE; 30 ng/kg per min) and L-NAME (50 μg/kg per min) were infused into the renal artery.
[a] $P < 0.01$.
[b] $P < 0.05$, compared with the corresponding basal value.
[c] $P < 0.01$.
[d] $P < 0.05$, compared with the corresponding control value.

4. Discussion

We have recently reported that a spontaneous NO donor NOC 7 attenuated antinatriuresis induced by norepinephrine and angiotensin II in anesthetized rabbits (Adachi et al., 1997). However, it is still unclear whether NOC 7 affects norepinephrine-induced hypofiltration or angiotensin II-evoked tubular reabsorption, because the influence of norepinephrine on filtration function was very small and the possible tubular action of angiotensin II was not observed in our study (Adachi et al., 1997). We had suggested that endogenous NO masks an ability of angiotensin II to enhance tubular reabsorption in anesthetized rabbits (Adachi et al., 1996). It is also likely, although not proven, that in the in vivo rabbit kidney endogenous NO counteracts with vascular and glomerular actions of norepinephrine, as suggested by Matsumura et al. (1995) in the dog kidney.

Considering the above-mentioned, the present study was performed to elucidate whether norepinephrine induces substantial hypofiltration and NOC 7 can attenuate the norepinephrine- and angiotensin II-induced antinatriuresis when the endogenous NO level is lowered with a NO synthase inhibitor L-NAME in anesthetized rabbits.

Intrarenal arterial infusion of norepinephrine reduced urine flow rate, urinary Na^+ excretion and fractional Na^+ excretion with slight reductions in renal blood flow and glomerular filtration rate in non-pretreated rabbits (group 1). Thus, the norepinephrine-induced antinatriuresis can be mainly related to the norepinephrine-evoked tubular reabsorption in NO-replete state.

L-NAME potentiated the norepinephrine-induced renal responses except the reduction in fractional Na^+ excretion (group 1). Norepinephrine reduced filtration fraction during L-NAME infusion. These results suggest that endogenous NO counteracts the norepinephrine-induced renal vasoconstriction mainly at the preglomerular site in the rabbit kidney, as demonstrated in the dog kidney (Matsumura et al., 1995). The norepinephrine-induced antinatriuresis may result from the hypofiltration response in the presence of L-NAME.

In L-NAME-pretreated rabbits (group 2), intrarenal arterial infusion of NOC 7 effectively attenuated norepinephrine-induced changes in renal blood flow, glomerular filtration rate, filtration fraction, urine flow rate and urinary Na^+ excretion. These results suggest that NOC 7 suppresses the norepinephrine-induced hypofiltration and thereby attenuates the antinatriuresis in the presence of L-NAME.

Our previous studies have shown that in anesthetized rabbits angiotensin II reduced renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion without affecting fractional Na^+ excretion in the absence of L-NAME (Adachi et al., 1996; Adachi et al., 1997) and that the angiotensin II-induced antinatriuresis was more pronounced with a reduction in fractional Na^+ excretion in the presence of L-NAME (Adachi et al., 1996). The angiotensin II-induced antinatriuresis, therefore, seems to be exclusively related to the reduced glomerular filtration in NO-replete state.

Also in the present study, angiotensin II caused renal vasoconstriction, hypofiltration and antinatriuresis with a reduction in fractional Na^+ excretion in L-NAME-pretreated rabbits (group 3). NOC 7 attenuated the angiotensin II-induced changes in urine flow rate, urinary Na^+ excretion and fractional Na^+ excretion but not the reductions in renal blood flow and glomerular filtration rate. These results suggest that NOC 7 can suppress the angiotensin II-evoked tubular reabsorption, but not the hypofiltration and thereby attenuates the antinatriuresis in the presence of L-NAME.

In contrast, our previous report showed that in L-NAME non-treated rabbits NOC 7 attenuated the norepinephrine-induced reduction in fractional Na^+ excretion and the angiotensin II-induced reduction in glomerular filtration rate (Adachi et al., 1997). Changes in the endogenous NO level may, thus, alter the site of interaction between the agonists and NOC 7. Since administration of NOC 7 (30 ng/kg per min) did not affect basal renal parameters (group 2 and 3), NOC 7 may not release sufficient amount of NO in the kidney. It could be therefore postulated that the reabsorption component may be more susceptible to NOC 7 than the filtration component in the angiotensin II-induced antinatriuresis (and the opposite is true in the norepinephrine-induced antinatriuresis) and that the released NO during NOC 7 infusion, which dose not elevate renal NO to the normal level, acts on the more susceptible site in the agonist-induced antinatriuresis when endogenous NO production is suppressed by L-NAME.

In conclusion, the present study suggests that in the rabbit kidney in vivo a NO donor NOC 7 can suppresses the norepinephrine-induced hypofiltration and the angiotensin II-evoked tubular reabsorption when the endogenous NO level is lowered by inhibition of NO synthase.

Acknowledgements

This work was supported by The Research Foundation for Pharmaceutical Sciences, Japan (to H.H.).

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