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Short communication

Renal effects of a nitric oxide donor, NOC 7, in anesthetized rabbits

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Abstract

Intrarenal arterial infusion of angiotensin II (4 ng/kg per min) reduced glomerular filtration rate and urinary Na^+ excretion without affecting fractional Na^+ excretion. Infusion of norepinephrine (30 ng/kg per min) reduced both urinary Na^+ excretion and fractional Na^+ excretion with a slight hypofiltration. The angiotensin II- and the norepinephrine-induced renal responses were suppressed during simultaneous infusion of a spontaneous nitric oxide donor 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl 1-triazene (NOC 7, 30 ng/kg per min) which itself had little influence on the renal parameters. The results suggest that in the rabbit kidney in vivo NOC 7 can interfere with the angiotensin II-induced hypofiltration and norepinephrine-evoked tubular reabsorption and thereby suppresses their antinatriuretic actions.

Keywords: Angiotensin II; Norepinephrine; NOC 7 (1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl 1-triazene); Nitric oxide (NO); Kidney; (Rabbit)

1. Introduction

Inhibitors of nitric oxide (NO) synthase have been reported to reduce renal blood flow, urine flow rate and urinary Na^+ excretion in experimental animals in vivo (Lahera et al., 1993; Salazar et al., 1992; Evans et al., 1994). The NO synthase inhibitors also enhance renal vasoconstriction and antinatriuresis induced by angiotensin II in rabbits (Adachi et al., 1996) and norepinephrine in dogs (Matsumura et al., 1995). Renal NO therefore may contribute to maintaining renal circulation and urine formation and protect the kidney against vasoconstrictor and antinatriuretic stimuli.

Studies using NO donors have also demonstrated the role of NO in the kidney. Sodium nitroprusside, which is metabolized to release NO at vascular smooth muscle cells (Bates et al., 1991), elicits vasodilation in the isolated perfused rat kidney (Heuzé-Joubert et al., 1992) and natriuresis in conscious rats (Grandes et al., 1991). There are also unique NO donors that can spontaneously release NO in biological fluids without necessity of co-factor or metabolic activation. *S*-Nitroso-*n*-acetylpenicillamine increases renal blood flow, urine flow rate, urinary Na^+ excretion and fractional excretion of Na^+ in anesthetized dogs pretreated with a NO synthase inhibitor (Majid et al., 1995). Spermine $N[O^-]N{=}O$ (NONOate) inhibits Na^+ reabsorption in the isolated perfused cortical collecting duct of the rat (Stoos et al., 1995).

In the present study, we examined whether a novel spontaneous NO donor, 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl 1-triazene (NOC 7), counteracts the angiotensin II- and norepinephrine-induced antinatriuresis 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 a continuous infu-

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sion of pentobarbital (2–4 mg/kg per h, i.v.) throughout the experiments. Inulin, dissolved in plasma extender solution (Hespander, Kyorin, Tokyo, Japan) 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 and measurement of blood pressure with a pressure transducer. The left kidney was exposed by a retroperitoneal flank incision, and the animal was suspended by clamping one of its lumbar spinous processes to facilitate the following 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. It should be noted that we did not examine whether this surgical procedure completely eliminates neural input to the kidney. 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 two side branches was placed into the renal artery for intrarenal arterial drug administration. After completion of surgery, more than 90 min were allowed for stabilization with continuous monitoring of urine flow rate and hemodynamics.

2.2. Experimental protocols

2.2.1. Group 1 (n = 6)

Urine was collected over a 10-min period and 1 ml of arterial blood was withdrawn at the midpoint of urine collection to obtain basal values. NOC 7 (Dozindo Laboratories, Kumamoto, Japan), dissolved in 0.01 M NaOH, was then infused into the renal artery at 10 ng/kg per min for 30 min. Twenty minutes after the start of NOC 7 infusion, 10-min urine sampling and blood sampling were performed, followed by experiments using sequentially higher doses (30 and 100 ng/kg per min) of NOC 7 in a similar manner. Sixty minutes after the end of NOC 7 infusion, the samples for recovery values were collected.

2.2.2. Group 2 (n = 7) and Group 3 (n = 6)

Urine and blood samples were collected to obtain basal values as in Group 1. Angiotensin II (Group 2, 4 ng/kg per min) or norepinephrine (Group 3, 30 ng/kg per min) was then infused into the renal artery for 15 min. Five minutes after the start of angiotensin II or norepinephrine infusion, the 10-min urine sampling and blood sampling were performed and the infusion was stopped. Ten minutes after the end of angiotensin II infusion, urine and blood samples for recovery values were collected. Then intrarenal arterial infusion of NOC 7 (30 ng/kg per min) was started. Twenty minutes after the start of NOC 7 infusion, a series of urine and blood sampling and angiotensin II or norepinephrine infusion was performed again.

2.3. Measurements

Blood samples were transferred to ice-chilled tubes containing EDTA and then centrifuged to obtain plasma samples. Glomerular filtration rate was determined as inulin clearance. Inulin concentration was measured by the anthrone method and Na^+ concentration by flame photometry. Urinary NO_x (NO_2^- and NO_3^-) level was determined by Griess reaction as reported by Süto et al. (1995) with slight modifications.

2.4. Statistics

All values are expressed as means ± S.E. Data for urine formation were transformed to logarithms to obtain normal distribution before application of statistical procedures. Statistical differences were evaluated by analysis of variance (ANOVA) for single-factor repeated measures and Dunnett's test (Group 1) or by ANOVA for multi-factor repeated measures and the simple main effects (Groups 2 and 3). Percentage changes in renal parameters induced by the agonists were compared between the control and the NOC 7 infusion periods by Student's paired *t*-test. Differences at $P < 0.05$ were considered to be statistically significant.

3. Results

Intrarenal arterial infusion of NOC 7 at either dose had no statistically significant effect on renal blood flow, glomerular filtration rate, urine flow rate, urinary Na^+ excretion or fractional Na^+ excretion except that mean arterial pressure decreased during NOC 7 infusion at 100 ng/kg per min (Group 1, Table 1). Urinary NO_X excretion increased during NOC 7 infusion at 10 and 30 ng/kg per min, but it returned to the basal level during the infusion at 100 ng/kg per min. Infusion of vehicle for NOC 7 (0.01 M NaOH, 0.1 ml/min) did not affect the renal or systemic parameters (data are not shown).

Fig. 1 shows the angiotensin II- and norepinephrine-induced renal responses as percentage changes from basal values. In the control period (before infusion of NOC 7), intrarenal arterial infusion of angiotensin II (Group 2) reduced renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion (by about 30%) without affecting fractional Na^+ excretion. Norepinephrine (Group 3) reduced urine flow rate and urinary Na^+ excretion and fractional Na^+ excretion (by about 25–35%) with minimal (about 10%) reductions in renal blood flow and glomerular filtration rate. After stopping the angiotensin II or norepinephrine infusion, each value recovered nearly to the basal level (data are not shown).

Intrarenal arterial infusion of NOC 7 (30 ng/kg per min) did not affect basal renal parameters (Groups 2 and

Table 1
Effects of NOC 7 on hemodynamics and renal functions (Group 1)

	Basal	NOC 7 (ng/kg per min)			Recovery
		10	30	100	
MAP (mmHg)	97 ± 3	97 ± 3	97 ± 4	93 ± 4 [a]	97 ± 4
RBF (ml/min)	26 ± 3	26 ± 3	25 ± 4	25 ± 4	23 ± 4
GFR (ml/min)	3.4 ± 0.2	3.2 ± 0.2	3.4 ± 0.3	3.4 ± 0.3	2.9 ± 0.4
UV (ml/min)	0.37 ± 0.08	0.41 ± 0.06	0.41 ± 0.06	0.34 ± 0.08	0.37 ± 0.08
UNaV (μEq/min)	29 ± 5	32 ± 4	33 ± 3	28 ± 4	28 ± 4
FENa (%)	6.1 ± 1.2	7.5 ± 1.0	7.2 ± 1.1	5.8 ± 0.6	7.1 ± 1.3
UNO_xV (nmol/min)	17 ± 3	21 ± 3 [a]	21 ± 2 [a]	18 ± 2	18 ± 2

Values are means ± S.E. $n = 6$. MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow rate; UNaV, urinary Na^+ excretion; FENa, fractional Na^+ excretion; UNO_xV, urinary NO_x excretion. NOC 7 was infused into the renal artery at the increasing doses.
[a] $P < 0.05$ compared with the corresponding basal value.

3). For example, the basal values in the control and the NOC 7 infusion periods in Group 2 were as follows: renal blood flow, 24 ± 2 and 21 ± 2 ml/min; glomerular filtration rate, 3.8 ± 0.4 and 3.2 ± 0.4 ml/min; urine flow rate, 0.32 ± 0.09 and 0.30 ± 0.07 ml/min; urinary Na^+ excretion, 26 ± 7 and 24 ± 5 μEq/min; fractional Na^+ excretion, 5.3 ± 1.3% and 5.7 ± 1.0%, respectively.

The changes in renal parameters induced by angiotensin II (reductions in renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion) and by norepinephrine (urine flow rate, urinary Na^+ excretion and fractional Na^+ excretion) were suppressed in the NOC 7 infusion period (Fig. 1).

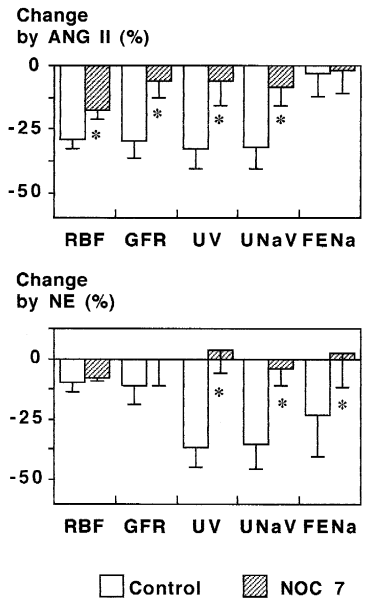

Fig. 1. Effects of NOC 7 on renal responses induced by angiotensin II (Group 2, upper panel) and norepinephrine (Group 3, lower panel). RBF, renal blood flow; GFR, glomerular filtration rate; UV, urine flow rate; UNaV, urinary Na^+ excretion; FENa, fractional Na^+ excretion. Values (means ± S.E.) are percentage changes from basal values in response to intrarenal arterial infusion of angiotensin II (ANG II, 4 ng/kg per min, $n = 7$) and of norepinephrine (NE, 30 ng/kg per min, $n = 6$) before and during intrarenal arterial infusion of NOC 7 (30 ng/kg per min). * $P < 0.05$ compared with the corresponding value obtained before the NOC 7 infusion (Control).

4. Discussion

In the present study, we examined effects of NOC 7 on antinatriuresis induced by angiotensin II and norepinephrine in anesthetized rabbits. NOC compounds are novel NO donors which are stable in alkaline solution and degraded to release NO in biological fluid (Hrabie et al., 1993).

Dose-response experiments were performed to determine the infusion rate of NOC 7 (Group 1). Intrarenal arterial infusion of NOC 7 at increasing rates of 10 and 30 ng/kg per min slightly elevated urinary NO_x excretion that has been used as an index for NO formation in vivo (Majid et al., 1995). Urine flow rate, urinary Na^+ excretion and fractional Na^+ excretion tended to increase during NOC 7 infusion, but the changes were not statistically significant. Renal and systemic hemodynamics remained unaffected. It is therefore possible that NOC 7 at these doses supplies NO to the kidney, but the amount of endogenous NO is sufficient to maintain basal renal hemodynamics and urine formation. The sequentially higher dose of NOC 7 (100 ng/kg per min) reduced mean arterial pressure, showing that this dose of NOC 7 induces systemic vasodilation despite the intrarenal arterial administration. Thus the intrarenal arterial infusion of NOC 7 even at the non-hypotensive doses could elevate NOC 7 level in the systemic circulation. NOC 7 at 100 ng/kg per min also reduced urinary NO_x excretion, which may result from enhanced tubular reabsorption of NO_X due to the hypotension as suggested by Süto et al. (1995) using sodium nitroprusside in conscious rats. Since the systemic hypotension complicates interpretation of results, NOC 7 was infused at 30 ng/kg per min in the following experiments (Groups 2 and 3).

Consistent with our previous results (Adachi et al., 1996), intrarenal arterial infusion of angiotensin II (Group

2) reduced renal blood flow, glomerular filtration rate, urine flow rate and urinary Na^+ excretion without a reduction in fractional Na^+ excretion. Therefore the angiotensin II-induced antinatriuresis may result from the reduced glomerular filtration, although angiotensin II can enhance renal tubular reabsorption (Harris and Navar, 1985). Endogenous NO may mask the tubular action of angiotensin II in the kidney of anesthetized rabbits (Adachi et al., 1996). The infusion of norepinephrine also reduced urine flow rate and urinary Na^+ excretion by the same degree as angiotensin II (Group 3). The dose of norepinephrine that causes the equipotent urinary responses had been determined in preliminary experiments. However, reductions in renal blood flow and glomerular filtration rate during norepinephrine infusion were smaller than the changes in urine flow rate and urinary Na^+ excretion, and a substantial reduction in fractional Na^+ excretion was observed. The results suggest that the norepinephrine-induced antinatriuresis is due predominantly to the enhanced tubular Na^+ reabsorption.

NOC 7 suppressed the angiotensin II-induced reductions in renal blood flow and glomerular filtration rate and the norepinephrine-induced reduction in fractional Na^+ excretion. The urine flow rate and urinary Na^+ excretion responses induced by these agonists were also suppressed in the presence of NOC 7. We had confirmed reproducibility of the renal responses induced by repetitive infusion of angiotensin II (Adachi et al., 1996) and of norepinephrine (preliminary experiments, data are not shown). Our present results suggest that NOC 7 can inhibit the renal vascular action of angiotensin II and the renal tubular action of norepinephrine, and thereby interfere with their antinatriuretic responses in the rabbit kidney in vivo. The inhibitory effects of NOC 7 could be related to its property as a NO releasing compound. The present study may provide further evidence for a protective role of NO against vasoconstrictor and antinatriuretic stimuli in the kidney.

In summary, the present study demonstrates that the angiotensin II- and norepinephrine-induced antinatriuresis, which may be due mainly to reduced glomerular filtration and enhanced tubular reabsorption, respectively, can be suppressed with a spontaneous NO donor, NOC 7, in anesthetized rabbits.

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