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Effects of furosemide on the tubular reabsorption of nitrates in anesthetized dogs

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Abstract

The present study was performed to determine the tubular sites of nitrite and nitrate (NO_x) reabsorption and the effects of furosemide on the renal handling of NO_x in anesthetized dogs, using renal clearance and stop-flow methods. Furosemide (2 mg/kg, i.v.) increased the urinary excretion rates of Na^+ ($U_{Na^+}V$) and NO_x ($U_{NO_x}V$) with a reduction of tubular reabsorption rates of Na^+ and NO_x. During inhibition of renal nitric oxide (NO) synthesis by an intrarenal infusion of L-nitro arginine (30 μg/kg-min), furosemide also increased $U_{NO_x}V$ and decreased tubular reabsorption rate of NO_x from 96.5 ± 0.8% to 86.6 ± 1.7%. An intravenous infusion of 10% mannitol (0.5 ml/kg-min) also increased both $U_{Na^+}V$ and $U_{NO_x}V$. In addition, after furosemide administration or mannitol infusion, $U_{NO_x}V$ was correlated with $U_{Na^+}V$. In stop-flow experiments, the distal dip in NO_x curve was observed and the site of the dip in NO_x curve was identical to that of Na^+ curve. Furosemide shifted upward the $U/P_{Na^+}/U/P_{Cr}$ and $U/P_{NO_x}/U/P_{Cr}$ at the distal dip, indicating inhibition of Na^+ and NO_x reabsorption at distal tubules. These results indicate that more than 96% of the filtered NO_x is reabsorbed in the renal tubules, and that the tubular handling of NO_x is very close to that of Na^+. In addition, the stop-flow experiments demonstrate that furosemide inhibited the reabsorption of NO_x as well as Na^+ at the distal tubule.

Keywords: Stop-flow experiment; Nitric oxide (NO); Nitrate; Distal, tubule; L-Nitro arginine

1. Introduction

Nitric oxide (NO) is produced by many types of mammalian cells and plays an important role in the regulation of systemic and local hemodynamics. NO has a short half-life, within a few seconds (Moncada et al., 1991; Ignarro, 1990), initially oxidised to nitrite (NO_2^-) (Ignarro, 1990; Hibbs et al., 1988) and then in the presence of oxyhemoglobin to nitrate (NO_3^-) (Kosaka et al., 1979; Ignarro et al., 1993). These circulating NO_2^- and NO_3^- (NO_x) are freely filtered through glomerulus (Wennmalm et al., 1993), reabsorbed in the renal tubule and a small portion of filtered NO_x is excreted into the urine (Godfrey and Majid, 1998; Suto et al., 1995). Since these nitrogen oxides are relatively stable in plasma and urine, plasma or urinary NO_x have been recently used as a marker of NO activity in vivo. Shultz and Raij (1992) reported increases in levels of NO_x in the serum and urine of rats treated with endotoxin. The increase in NO_x levels was inhibited by coadministration of the NO synthase inhibitor, confirming that the NO_x measured in the serum and urine after endotoxin reflected changes in NO production. Thus, they suggested that the urinary excretion rate of NO_x ($U_{NO_x}V$) may be useful as an indicator of endogenous NO activity in vivo. On the other hand, a study by Suto et al. (1995) has questioned whether the acute change in $U_{NO_x}V$ reflects the systemic and/or renal NO production. They showed that a systemic administration of L-arginine activating NO synthesis resulted in a significant increase in $U_{NO_x}V$ in conscious rats, but D-arginine also increased the $U_{NO_x}V$ to the same degree as with L-arginine. Based on these findings, they suggested that the $U_{NO_x}V$ does not reflect the acute activity of the systemic and/or renal NO production.

The recent work using anesthetized dogs by Godfrey and Majid (1998) showed that NO_x is extensively reab-

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sorbed and $U_{NO_x}V$ increases progressively in response to increase in its circulating levels without exhibiting a transport maximum. These authors, Godfrey and Majid (1998) and Suto et al. (1995), have suggested that tubular site for NO_x reabsorption may be a proximal tubule. However, Majid et al. (1995) reported that $U_{NO_x}V$ correlates with $U_{Na^+}V$. As cited previously, Suto et al. (1995) reported both L- and D-arginine increase $U_{NO_x}V$ along with diuresis. Taken together, it can be considered that NO_x reabsorption may depend on Na^+ reabsorption. We hypothesized that NO_x along with Na^+ should be reabsorbed in the distal tubule as well as in the proximal tubule. Thus, the present study was performed to determine the tubular sites of NO_x reabsorption and the effects of furosemide or mannitol which have the differing action site and mechanism, on the renal handling of NO_x in anesthetized dogs, using clearance and stop-flow methods.

2. Materials and methods

2.1. General procedures

Experiments were carried out on adult mongrel dogs weighing 10–16 kg, which had been maintained on standard laboratory chow for 1 week. All surgical and experimental procedures were performed according to the guidelines for care and use of animals as established by the Kagawa Medical University. The animals were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and were given additional doses, as required. Animals were intubated with a cuffed endotracheal tube and artificially ventilated (Harvard apparatus ventilator; Harvard Apparatus, Millis, MA). Catheters were inserted into the right brachial vein and artery for infusion of saline or drug solution and for arterial blood sampling, respectively. Another catheter was placed in the abdominal aorta via the right femoral artery and mean blood pressure was continuously monitored with a pressure transducer. The left kidney was exposed by a retroperitoneal flank incision and renal blood flow was continuously measured by placing an electromagnetic flow probe (Nihonkohden, Tokyo, Japan) around the renal artery. The left ureter was catheterized with a polyethylene tube for the collections of urine samples. After completion of the surgical preparation, an intravenous infusion of 2.5 to 3.0 ml/min of 0.9% saline was started. To measure the glomerular filtration rate, the priming dose of creatinine (100 mg/kg) was administered into the right brachial vein, followed by a maintenance dose of creatinine (50 mg/kg h) dissolved in isotonic saline and the dog was left for 60–90 min to allow for stabilization of mean blood pressure, renal blood flow, and urine flow. After the completion of experiment, the kidney was removed and weighed. All calculated parameters were expressed per gram of kidney mass in the present experiment.

2.2. Clearance experiments

After equilibration, urine was collected over two consecutive 10-min control clearance periods. After the second control period, furosemide was administered intravenously at a dose of 2 mg/kg in five dogs and 3–5 min was allowed for stabilization before sampling. Then, urine was collected during two consecutive 10-min clearance periods. Arterial blood samples were obtained at a midpoint of each period. In another six dogs, after two control clearance periods, the L-nitro arginine (LNA) was infused into the renal artery at a rate of 30 μg/kg-min and 1 h after starting LNA infusion, the same above protocol was repeated to examine the effects of furosemide during inhibition of NO synthase. In five other dogs, after collection of two blood and urine samples, 10% mannitol in isotonic saline was infused intravenously at a rate of 0.5 ml/kg-min. When urine flow had been stabilized at a high level (usually 40–45 min after starting mannitol infusion), urine

Table 1
Effects of furosemide or mannitol on renal hemodynamics and plasma concentrations of NO_2/NO_3 (NO_x) in anesthetized dogs

	MBP (mm/Hg)	RBF (ml/min · g)	GFR (ml/min · g)	Urine flow (μl/min · g)	$U_{Na^+}V$ (μmol/min · g)	Plasma NO_x (μM)
Control	115 ± 8	3.00 ± 0.71	0.61 ± 0.10	10 ± 3	1.2 ± 0.6	27.4 ± 4.4
Furosemide (2 mg/kg, i.v.)						
10 min	129 ± 7[a]	4.06 ± 0.33	0.65 ± 0.09	161 ± 35[a]	20.8 ± 4.6[a]	24.9 ± 4.3
20 min	129 ± 6[a]	3.68 ± 0.37	0.69 ± 0.06	162 ± 25[a]	20.3 ± 3.4[a]	29.3 ± 5.1
Control	121 ± 3	2.49 ± 0.24	0.70 ± 0.11	10 ± 3	1.2 ± 0.7	29.6 ± 4.2
Mannitol (10% mannitol solution was infused intravenously at a rate of 0.5 ml/kg-min)						
50 min	133 ± 7	2.92 ± 0.45	0.56 ± 0.12	101 ± 24[a]	8.8 ± 2.7[a]	25.0 ± 3.0
60 min	133 ± 7	2.93 ± 0.44	0.54 ± 0.13	101 ± 26[a]	8.8 ± 3.0[a]	29.0 ± 4.1

All values are means ± S.E.
MBP: mean blood pressure, RBF: renal blood flow, GFR: glomerular filtration rate, $U_{Na^+}V$: urinary excretion rate of Na^+, Plasma NO_x: plasma concentration of NO_x.
[a] $P < 0.05$ vs. control.

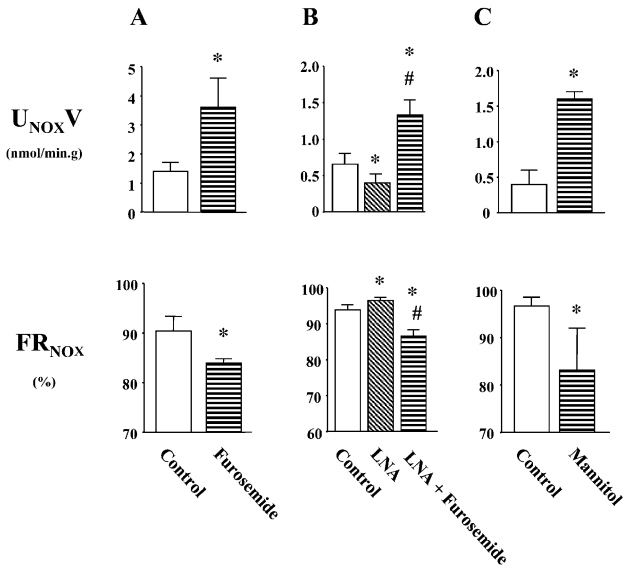

Fig. 1. Effects of furosemide (2 mg/kg, i.v.) or mannitol on the urinary excretion rates of nitrite and nitrate (NO_x) ($U_{NO_x}V$) and fractional reabsorption of NO_x (FR_{NO_x}) in anesthetized dogs. (A) Furosemide alone ($N = 5$), (B) furosemide with the pretreatment of L-nitro arginine (LNA, $N = 6$), (C) mannitol ($N = 5$). $^{*}P < 0.05$ vs. control and $^{\#}P < 0.05$ vs. LNA.

and blood samples were obtained during two consecutive 10-min clearance periods.

2.3. Stop-flow experiments

The stop-flow experiments were conducted in five dogs according to the method described by Malvin and Wilde (1973). After the surgery was completed, an initial dose of *p*-aminohippuric acid (50 mg/kg) and creatinine (100 mg/kg) were given intravenously followed by a constant intravenous infusion of 10% mannitol solution containing *p*-aminohippuric acid (0.1%) and creatinine (0.17%) at a rate of 0.5 ml/kg-min. When urine flow had stabilized at a high level, two consecutive 6-min urine samples were obtained and arterial blood sample was collected at a midpoint of each period. These samples were considered as free flow control samples. After collection of free flow samples, the left ureteral catheter was clamped near its tip for 6 min. Just after 6 min of occlusion, the obstruction was released and the rapidly flowing urine was collected serially into 25 polyethylene tubes each of approximately 0.5 ml urine volume. After at least a 60-min recovery period, furosemide was administered intravenously at a dose of 2 mg/kg. Five minutes after administration of furosemide, two 6-min free flow urine and blood samples were obtained. After the collection of the second free-flow samples, the second stop-flow procedures were carried out on the same animal.

2.4. Analytical methods

Urine and plasma creatinine were measured by colorimetry according to the Jaffe reaction (Bonsnes and Taussky, 1945) and *p*-aminohippuric acid by the Bratton–Marshall method (Fister, 1950). Na^+ in plasma and urine was determined by flame photometry (Instrumentation Laboratory, model 143), and NO_x was measured by the Griess method after reduction of NO_3^- to NO_2^- in the cadmium column (Green et al., 1982). Glomerular filtration rate was estimated by creatinine clearance. *p*-Aminohippuric acid was used to distinguish the proximal fluid in stop-flow experiments.

2.5. Statistical analysis

The values were presented as means ± S.E. Statistical analysis was performed by Student's paired *t*-test. Correlation of the responses was made by the Spearman test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of furosemide on renal hemodynamics and handling of NO_x with and without LNA

The intravenous administration of furosemide (2 mg/kg) significantly increased urine flow and urinary excre-

Table 2
Effects of furosemide on renal hemodynamics and plasma concentrations of NO_2/NO_3 (NO_x) during LNA infusion

	MBP (mm/Hg)	RBF (ml/min · g)	GFR (ml/min · g)	Urine flow (μl/min · g)	$U_{Na^+}V$ (μmol/min · g)	Plasma NO_x (μM)
Control	125 ± 5	2.85 ± 0.73	0.78 ± 0.11	16 ± 5	1.9 ± 0.7	19.2 ± 5.1
LNA (30 μg/kg-min, intrarenal infusion)						
LNA 70 min	144 ± 8[a]	1.90 ± 0.54[a]	0.67 ± 0.13[a]	8 ± 3[a]	0.7 ± 0.3[a]	21.2 ± 4.0
LNA 80 min	146 ± 8[a]	1.89 ± 0.53[a]	0.72 ± 0.15	10 ± 4[a]	0.9 ± 0.5[a]	19.9 ± 3.4
Furosemide (2 mg/kg, i.v.) during intrarenal infusion of LNA (30 μg/kg-min)						
Furosemide 10 min	143 ± 7	2.59 ± 0.82[b]	0.66 ± 0.15	108 ± 32[b]	13.9 ± 4.2[b]	18.9 ± 3.4
Furosemide 20 min	143 ± 6	2.31 ± 0.78	0.59 ± 0.12	102 ± 28[b]	12.7 ± 3.5[b]	20.9 ± 4.1

All values are means ± S.E ($N = 6$).
Abbreviations: LNA: L-nitro arginine. and see legends in Table 1.
[a] $P < 0.05$ vs. control.
[b] $P < 0.05$ vs. LNA 80 min.

tion rate of Na^+ ($U_{Na^+}V$) without any changes in renal blood flow and glomerular filtration rate (Table 1). As shown in Fig. 1A, the $U_{NO_x}V$ significantly increased from 1.4 ± 0.3 to 3.6 ± 1.0 nmol/min · g and the fractional reabsorption of NO_x (FR_{NO_x}) decreased from $90.4 \pm 2.9\%$ to $83.9 \pm 0.9\%$. Plasma concentration of NO_x was not affected. The intrarenal infusion of LNA (30 μg/kg-min) increased mean blood pressure and decreased renal blood

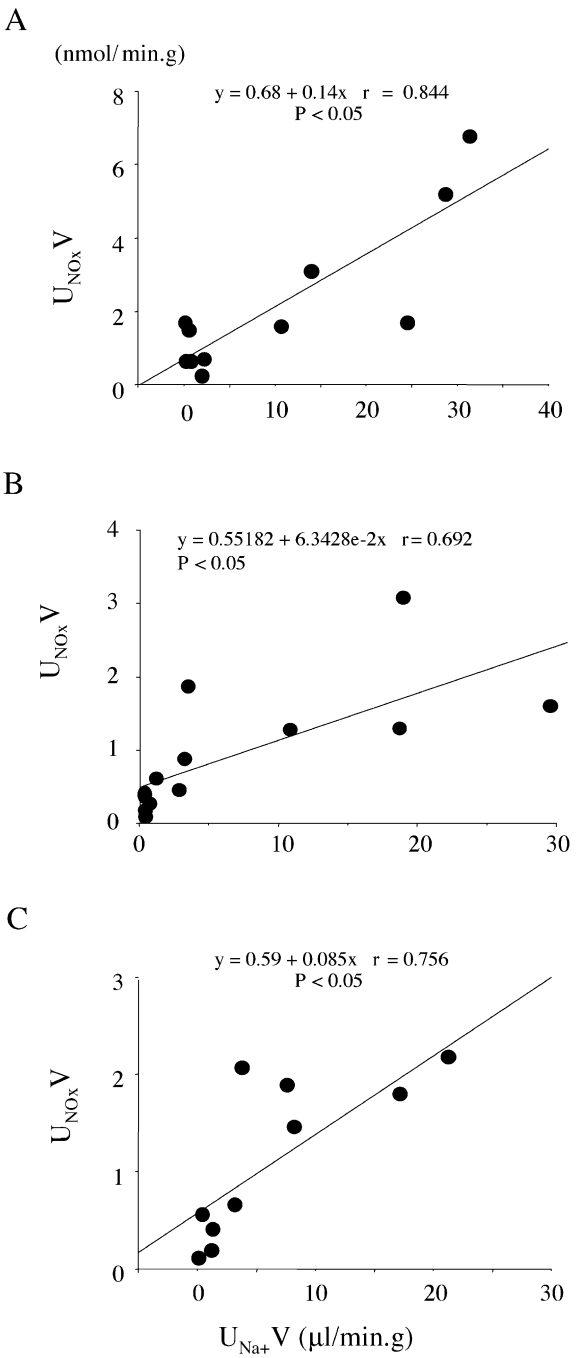

Fig. 2. The relationship between the urinary excretion rates of NO_x ($U_{NO_x}V$) and of Na^+ ($U_{Na^+}V$). (A) Furosemide alone ($N = 5$), (B) furosemide with the pretreatment of L-nitro arginine (LNA, $N = 6$), (C) mannitol ($N = 5$).

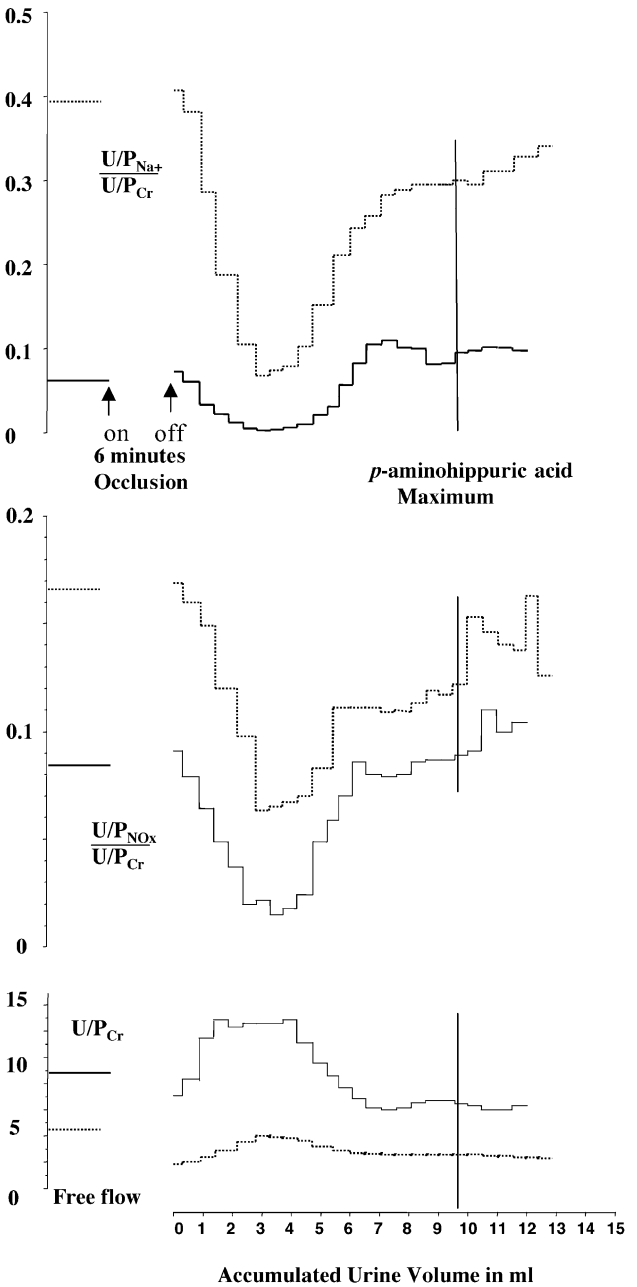

Fig. 3. The effects of mannitol (solid line) and furosemide plus mannitol (dotted line) on the stop-flow pattern of Na^+ and NO_x in the anesthetized dog.

flow, urine flow and $U_{Na^+}V$ (Table 2). $U_{NO_x}V$ decreased from 0.65 ± 0.15 to 0.40 ± 0.12 nmol/min · g, indicating the inhibition of renal synthesis of NO (Fig. 1B). During infusion of LNA, furosemide (2 mg/kg, i.v.) significantly increased $U_{NO_x}V$ from 0.40 ± 0.12 to 1.33 ± 0.23 nmol/min · g and decreased the FR_{NO_x} from $96.5 \pm 0.8\%$ to $86.6 \pm 1.7\%$. These effects of furosemide on $U_{NO_x}V$ and FR_{NO_x} did not differ between treatment and non-treatment of LNA. In addition, since furosemide did not affect the plasma concentration of NO_x or glomerular filtration rate in both groups, the filtered load of NO_x also did not

change. Thus, these results clearly indicate that furosemide inhibited the tubular reabsorption of NO_x.

The intravenous infusion of 10% mannitol increased urine flow, $U_{Na^+}V$ and $U_{NO_x}V$ without any change in glomerular filtration rate (Table 1 and Fig. 1C). Plasma concentration of NO_x slightly decreased during mannitol infusion, but it was not statistically significant. These results indicate that mannitol inhibited the tubular reabsorption of NO_x.

Fig. 2 shows the correlation between $U_{Na^+}V$ and $U_{NO_x}V$ after the administration of furosemide alone, furosemide with LNA or during the mannitol infusion. In all groups, $U_{NO_x}V$ are well correlated with $U_{Na^+}V$. These results indicate that the renal handling of NO_x has been very close to that of Na^+.

3.2. Effects of furosemide on the stop-flow pattern of sodium and NO_x

The stop-flow experiments were performed in five dogs. However, since similar stop-flow patterns were obtained in all five dogs, the results of representative experiments are reported here. The stop-flow patterns of Na^+ and NO_x before and after the administration of furosemide are illustrated in Fig. 3. The proximal tubular location is estimated by the urine sample with the maximum relative ratio of *p*-aminohippuric acid (PAH) urine (U)/plasma (P) to creatinine U/P ($U/P_{PAH}/U/P_{Cr}$) and indicated as the *p*-aminohippuric acid maximum at 9.8 ml on horizontal scale in Fig. 3. The ratio of Na^+ U/P (U/P_{Na^+}) to U/P_{Cr} ($U/P_{Na^+}/U/P_{Cr}$) and the ratio of NO_x U/P to U/P_{Cr} ($U/P_{NO_x}/U/P_{Cr}$) in the urine collected immediately before the first occlusion were 0.060 and 0.085, respectively, indicating net tubular reabsorption of them. However, both $U/P_{Na^+}/U/Pcr$ and $U/P_{NO_x}/U/Pcr$ started to decrease along with the accumulated urine volume on the horizontal scale and reached the respective minimum level at 3.3 ml of accumulated urine volume, and then progressively increased to the respective basal value. Thus, the stop-flow curve of Na^+ clearly shows the early dip, so-called distal dip, which is considered to represent the tubular fluid in the distal area. In addition, the distal dip in the NO_x curve was also observed and the site of the dip in the NO_x curve was identical to that in the Na^+ curve. The administration of furosemide (2 mg/kg, i.v.) during mannitol diuresis resulted in a further diuresis accompanied by an increased $U_{NO_x}V$ (Table 3) and an upward shift of both the Na^+ and NO_x curves (Fig. 3). The $U/P_{Na^+}/U/P_{Cr}$ and $U/P_{NO_x}/U/P_{Cr}$ at the distal dip after furosemide administration increased from 0.003 and 0.021 to 0.074 and 0.073, respectively, indicating the inhibition of Na^+ and NO_x reabsorption at distal tubules.

4. Discussion

The aim of the present experiment is to determine the tubular sites of NO_x reabsorption and to investigate the effects of furosemide on the renal handling of NO_x in anesthetized dogs. Both furosemide and mannitol significantly increased the $U_{NO_x}V$ and decreased the FR_{NO_x}. These effects of furosemide were also observed during the inhibition of intrarenal NO synthesis. These results suggest that furosemide induced increase of $U_{NO_x}V$ mainly due to the inhibition of NO_x reabsorption. Furthermore, the results of stop-flow experiments clearly showed that NO_x was reabsorbed at the distal tubules, and that furosemide inhibited the NO_x reabsorption as well as Na^+ reabsorption at the distal site of renal tubules.

In the present study, we chose two classic methods, clearance and stop-flow methods, to determine the renal handling of NO_x. To minimize the dietary contribution of NO_x levels in plasma and urine, dogs were fasted for 24 h before starting the experiment, since it has been reported that 24 h of fasting reduces the plasma NO_x level to nearly 80% (Suzuki et al., 1992). However, since the kidney synthesizes and release NO and NO_x is freely filtered, urinary NO_x levels reflect both systemic and renal production of NO. Then, to avoid the reflection of renal production of NO, we have compared the effects of furosemide on renal handling of NO_x with and without the inhibition of renal synthesis of NO. The intrarenal infusion of LNA decreased $U_{NO_x}V$ without any changes in the filtered load of NO_x, indicating the inhibition of renal NO production. The FR_{NO_x} was around 96%. These results are consistent with those in the report by Godfrey and Majid (1998).

Table 3
Effects of furosemide on renal hemodynamics and plasma concentrations of NO_2/NO_3 (NO_x) during mannitol diuresis

	MBP (mm/Hg)	RBF (ml/min · g)	GFR (ml/min · g)	Urine flow (μl/min · g)	$U_{Na^+}V$ (μmol/min · g)	FR_{Na^+} (%)	$U_{NO_x}V$ (nmol/min · g)	FR_{NO_x} (%)
Mannitol	131 ± 8	2.65 ± 0.28	0.63 ± 0.14	142 ± 20	11.7 ± 2.3	86.1 ± 3.3	1.9 ± 0.4	83.6 ± 7.3
Intravenous administration of furosemide (2 mg/kg) during 10% mannitol (0.5 ml/kg-min, i.v.) infusion								
Furosemide 10 min	126 ± 7	3.61 ± 0.33[a]	0.69 ± 0.19	293 ± 26[a]	33.6 ± 3.7[a]	60.6 ± 6.8[a]	3.3 ± 0.5[a]	74.3 ± 8.1[a]
Furosemide 20 min	126 ± 7	3.62 ± 0.34[a]	0.75 ± 0.24	292 ± 23[a]	36.7 ± 4.8[a]	59.9 ± 6.9[a]	3.3 ± 0.5[a]	72.6 ± 11.5[a]

All values are means ± S.E. ($N = 5$).

Abbreviations: FR_{Na^+}: fractional reabsorption rate of Na^+, FR_{NO_x}: fractional reabsorption rate of NO_x and see legends in Table 1 or Table 2.

[a] $P < 0.05$ vs. mannitol.

During inhibition of renal NO synthesis, furosemide increased the $U_{NO_x}V$ by threefold. There were no differences between the effects of furosemide on renal handling of NO_x with and without the inhibition of renal NO synthesis. Thus, these results suggest that the renal and systemic production of NO may partly affect the $U_{NO_x}V$. However, since the FR_{NO_x} is more than 90%, the tubular reabsorption of NO_x seems to be a more important determinant of the $U_{NO_x}V$.

Suto et al.(1995) have reported that D-arginine increased $U_{NO_x}V$ along with diuresis, and these effects are not different from those by L-arginine. These findings indicate that $U_{NO_x}V$ might be dependent on the Na^+ handling in the renal tubules rather than a reflection of systemic or renal synthesis of NO. As shown in Fig. 2, there was a good correlation between $U_{Na+}V$ and $U_{NO_x}V$ in all experimental groups. These results may indicate that the renal handling of NO_x is very close to that of Na^+.

It is well recognized that mannitol functions as a diuretic by virtue of its osmotic action in the proximal tubule, whereas furosemide acts as a diuretic mainly on the thick ascending limb of the loop of Henle. Thus, although both agents have different mechanisms and action sites as diuretics, they exerted nearly the same effect on the renal handling of NO_x, indicating that NO_x might be reabsorbed in all segments of renal tubule. The superimposition of furosemide on mannitol resulted in a further increase of $U_{NO_x}V$. These results might support the above speculation. On the other hand, Suto et al. (1995) have reported that furosemide (1 mg/kg, i.v.) had little effect on $U_{NO_x}V$ in conscious rats. The difference between the study by Suto et al. and our study may have been due to dose-dependant action of furosemide, differences in experimental condition or to species difference.

The present results showing that furosemide as well as mannitol increased the $U_{NO_x}V$ may indicate that NO_x is also reabsorbed at the distal sites of renal tubules. To determine whether NO_x is reabsorbed even at the distal tubules, stop-flow experiments were conducted. As shown in Fig. 3, the so-called distal dip was observed in the control curve of NO_x, indicating that NO_x was markedly reabsorbed at the distal tubules. Interestingly, the site of the dip of NO_x curve was identical to that of the Na^+ curve. Thus, these findings clearly indicate that NO_x was reabsorbed at the tubules which intensely reabsorbed Na^+, and that both NO_x and Na^+ were concomitantly reabsorbed at the same tubular sites. Furosemide shifted upward both NO_x and Na^+ curves. The rise of $U/P_{Na^+}/U/P_{Cr}$ and $U/P_{NO_x}/U/P_{Cr}$ at the distal dip may indicate the inhibitory effects of furosemide on Na^+ and NO_x reabsorption at the distal tubules. The $U/P_{Na^+}/U/P_{Cr}$ and $U/P_{NO_x}/U/P_{Cr}$ at the proximal area were also shifted upward, indicating the inhibitory action of furosemide even at the proximal tubules. However, since the tubular fluid at the proximal site should pass through the distal tubule after the release of the ureteral clamp and is modified by the distal tubule, it is difficult to make a conclusion about the drug effects on the proximal tubule from the stop-flow pattern (Orloff, 1966).

Mundel et al. (1992) have reported that a brain NO synthase mRNA is expressed abundantly in macula densa cells. Therefore, we expected to find peaks in the NO_x curve. However, we could not find any peak in the NO_x curve and unexpectedly found a distal dip. These findings indicate that the tubular reabsorption of NO_x may overcome the renal synthesis and release of NO_x. Thus, the present stop-flow experiment also clearly shows that NO_x was reabsorbed powerfully at the distal tubule and furosemide inhibited the reabsorption of NO_x as well as Na^+ at the distal tubule. Majid et al. (1995) have reported that the distal diuretics, thiazide or amiloride did not affect $U_{NO_x}V$ in anesthetized dogs. Although these authors did not examine the effects of furosemide on the $U_{NO_x}V$, it is interesting that there are quite discrepancies between their results and our results. Based on the present results, we are unable to explain the above differences and to define the transport mechanisms of NO_x. However, it is now evident that NO_x is reabsorbed powerfully at the distal tubules and may be reabsorbed concomitantly with Na^+.

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