Effect of Norepinephrine on the Urinary Excretion of Purine Bases and Oxypurinol

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To examine whether norepinephrine affects the plasma concentrations and urinary excretion of purine bases and oxypurinol, we orally administered allopurinol (300 mg) to 5 healthy subjects and 9 hours later intravenously administered norepinephrine (12 to 20 μg/kg body weight), which causes a more than 10 mm Hg increase in diastolic pressure for 2 hours. Norepinephrine decreased the urinary excretion of uric acid by 33% ($P < .01$), oxypurinol by 32% ($P < .01$), and xanthine by 51% ($P < .01$), as well as the fractional clearance of uric acid by 32% ($P < .01$), oxypurinol by 24% ($P < .05$), and xanthine by 21% ($P < .05$) when measured 1 to 2 hours after administration. These results indicate that norepinephrine decreases the urinary excretion of uric acid, oxypurinol, and xanthine, probably via hemodynamic change. It is also suggested that the hypouricemic effect of allopurinol may be more potent than that expected in gout patients with enhanced sympathetic tone, such as in salt-sensitive hypertension.

NOREPINEPHRINE IS A vasoconstrictor that is used for the treatment of shock from myocardial infarction, as well as sepsis, anaphylaxy, and bleeding. It is released from terminal boutons of the sympathetic nerve and elevates blood pressure via vascular constriction. Norepinephrine is physiologically released via increased sympathetic tone, which is induced by emotional stress or exercise, leading to construction of vessels. Previously, it was demonstrated that norepinephrine decreases the urinary excretion of uric acid, and this decreased urinary excretion was suggested to be dependent on the reduction in renal blood flow caused by norepinephrine.[1]

Many reports[2-6] have suggested that uric acid partially shares a renal transport pathway with xanthine and oxypurinol. A recent study[6] demonstrated that furosemide decreased the renal clearance of uric acid, xanthine, and oxypurinol, also suggesting the presence of this common renal pathway. The results of these previous studies led us to speculate that norepinephrine may decrease the clearance of oxypurinol, as well as uric acid. Oxypurinol is a metabolite of allopurinol, which is used for the treatment of hyperuricemia. Although both allopurinol and oxypurinol are potent inhibitors of xanthine oxidase, the biologic half-life of oxypurinol is longer than that of allopurinol. Accordingly, the overall effect of allopurinol may depend on the action of oxypurinol.[7-9] If norepinephrine decreases the urinary excretion of oxypurinol, the concentration of oxypurinol in plasma may increase. This finding would be clinically important, because the hypouricemic effect of allopurinol mostly depends on plasma oxypurinol levels. Therefore, to determine whether norepinephrine decreases the renal clearance of oxypurinol together with that of purine bases (hypoxanthine, xanthine, and uric acid), we conducted the present study by increasing the plasma level of norepinephrine (12 to 20 μg/kg body weight), which causes a more than 10 mm Hg increase in diastolic pressure.

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SUBJECTS AND METHODS

Chemicals

Allopurinol was purchased from GlaxoWellcome Japan (Tokyo, Japan). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Subjects and Protocol

Five men, aged 37 to 45 years old (body weight, 52 to 68 kg), each with normal laboratory data, participated in the study after informed consent was obtained. The study protocol is shown in Fig 1. In brief, allopurinol (300 mg) was administered orally to the subjects at 11:30 PM after a 4-hour fast. At 8.30 AM the next day, their urine was completely voided and the first 1-hour urine samples were collected (first period). After the first urine samples were collected, norepinephrine (12 to 20 μg/kg body weight), which causes a more than 10 mm Hg increase in diastolic pressure, was administered intravenously over 2 hours. The infusion rate was adjusted by measuring blood pressure every 10 minutes. The second urine samples were collected 1 hour after beginning the administration of norepinephrine (second period), and the third urine samples were collected between 1 and 2 hours after beginning the administration of norepinephrine (third period). The first, second, and third blood samples were drawn with heparinized syringes at the midpoint of the respective 1-hour urinary collections. Blood pressure, described in the Results, was measured just prior to each blood drawing. Two weeks later, a control study was performed using the same protocol, except without the administration of norepinephrine. Both studies were performed with a fast, except for water.

Blood and Urine Analyses

The concentrations of hypoxanthine, xanthine, and oxypurinol in plasma and urine were determined using high-performance liquid chromatography (HPLC), as described previously.[5] In brief, the column was a Wakosil 5C-18-200 (4.6 × 250 mm) (Wako Pure Chemical Industries), with a flow rate of 1 mL/min and a mobile phase of 0.02 mol/L KH_2PO_4 (pH 2.2). To measure the plasma concentrations of hypoxanthine, xanthine, and oxypurinol, plasma was immediately separated after drawing blood with a heparinized syringe. The concentrations of uric acid and creatinine in plasma and urine were measured by the uricase method using a Uric Acid B Test Wako Kit (Wako Pure Chemical Industries) and by the enzymatic method using a Diacolor Liquid CRE Kit (Toyobo, Osaka, Japan), respectively. Plasma norepinephrine was measured by Special Research Laboratories (Tokyo, Japan), while others were measured in our hospital laboratory. The

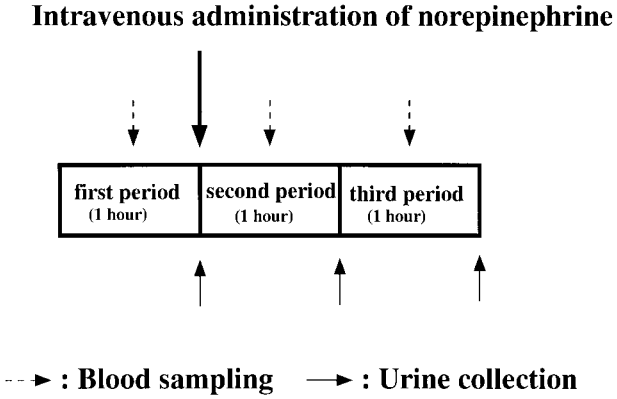

Fig 1. Study protocol.

percentage ratios of uric acid clearance/creatinine clearance (fractional uric acid clearance), hypoxanthine clearance/creatinine clearance (fractional hypoxanthine clearance), xanthine clearance/creatinine clearance (fractional xanthine clearance), and oxypurinol clearance/creatinine clearance (fractional oxypurinol clearance) were calculated.

Statistical Analysis

Data are presented as the mean ± SD. The significance of difference between variables was analyzed by analysis of variance (ANOVA).

RESULTS

Effect of Norepinephrine Administration on the Urinary Excretion of Purine Bases and Oxypurinol, and Urine Output

In the norepinephrine loading study, urinary output did not change significantly (Table 1). The urinary excretion of uric acid, oxypurinol, and xanthine decreased by 27% ($P < .01$), 28% ($P < .05$), and 36% ($P < .05$), respectively, in the second period, and 33% ($P < .01$), 32% ($P < .01$), and 51% ($P < .01$), respectively, in third period, as compared with the same in the first period (Table 1). However, the urinary excretion of hypoxanthine and creatinine was not changed. On the other hand, the respective values did not change significantly in the control study (Table 1).

Effect of Norepinephrine Administration on the Plasma Concentrations of Purine Bases and Oxypurinol

In the norepinephrine loading study, as well as in the control study, the plasma concentrations of uric acid, xanthine, hypoxanthine, and oxypurinol did not change significantly, nor did that of creatinine throughout the study (Table 2).

Effect of Norepinephrine Administration on the Fractional Clearance of Purine Bases and Oxypurinol and the Clearance of Creatinine

In the norepinephrine loading study, the fractional clearance of uric acid and xanthine decreased by 26% ($P < .01$) and 20% ($P < .05$), respectively, in the second period and that of uric acid, xanthine, and oxypurinol decreased by 32% ($P < .01$), 21% ($P < .05$), and 24% ($P < .05$), respectively, in the third period (Table 3) as compared with the same in the first period. However, the fractional clearance of hypoxanthine and the clearance of creatinine did not change significantly. In the control study, the fractional clearance of uric acid, oxypurinol, hypoxanthine, and xanthine, as well as the clearance of creatinine, did not change significantly (Table 3).

Table 1. Urinary Excretion of Purine Bases and Oxypurinol (N = 5)

	1st Period	2nd Period	3rd Period
Norepinephrine loading study			
Urine volume (mL/h)	254 ± 55	272 ± 68	261 ± 58
Hypoxanthine (μmol/h)	11.39 ± 5.14	8.26 ± 3.39	6.82 ± 4.26
Xanthine (μmol/h)	20.37 ± 7.38	13.08 ± 3.48*	9.91 ± 3.02†
Uric acid (μmol/h)	176 ± 20	129 ± 20†	118 ± 20†
Oxypurinol (μmol/h)	33.07 ± 4.34	23.65 ± 6.60*	22.64 ± 3.93*
Creatinine (mmol/h)	0.56 ± 0.07	0.55 ± 0.06	0.55 ± 0.06
Control study			
Urine volume (mL/h)	180 ± 58	205 ± 65	177 ± 59
Hypoxanthine (μmol/h)	10.70 ± 3.66	8.70 ± 2.68	7.00 ± 2.51
Xanthine (μmol/h)	20.18 ± 5.89	18.20 ± 5.52	16.31 ± 4.48
Uric acid (μmol/h)	167 ± 22	168 ± 20	171 ± 18
Oxypurinol (μmol/h)	32.66 ± 5.57	31.84 ± 4.88	31.41 ± 5.31
Creatinine (mmol/h)	0.60 ± 0.03	0.59 ± 0.03	0.59 ± 0.02

NOTE. Values are expressed as mean ± SD; 1st period, 2nd period, and 3rd period are described in Fig 1.
*$P < .05$.
†$P < .01$.

Effect of Norepinephrine Administration on Plasma Norepinephrine and Blood Pressure

Plasma norepinephrine increased by 11.0-fold ($P < .01$) and 11.2-fold ($P < .01$) in the second and third periods, respectively, while systolic and diastolic arterial blood pressure in-

Table 2. Plasma Concentrations of Purine Bases and Oxypurinol (N = 5)

	1st Period	2nd Period	3rd Period
Norepinephrine loading study			
Hypoxanthine (μmol/L)	1.42 ± 0.44	1.14 ± 0.30	0.98 ± 0.22
Xanthine (μmol/L)	2.94 ± 1.04	2.52 ± 1.00	1.88 ± 0.70
Uric acid (μmol/L)	274 ± 33	280 ± 30	274 ± 32
Oxypurinol (μmol/L)	23.04 ± 2.80	21.98 ± 2.50	21.74 ± 2.58
Creatinine (μmol/L)	84 ± 7	84 ± 6	84 ± 5
Control study			
Hypoxanthine (μmol/L)	1.42 ± 0.36	1.22 ± 0.26	1.04 ± 0.22
Xanthine (μmol/L)	2.86 ± 0.80	2.52 ± 0.76	2.18 ± 0.68
Uric acid (μmol/L)	280 ± 39	270 ± 37	274 ± 35
Oxypurinol (μmol/L)	23.90 ± 2.22	22.96 ± 2.10	22.34 ± 2.14
Creatinine (μmol/L)	84 ± 5	84 ± 7	84 ± 7

NOTE. Values are expressed as mean ± SD; 1st period, 2nd period, and 3rd period are described in Fig 1.
*$P < .05$.

Table 3. Fractional Clearance of Purine Bases and Oxypurinol (N = 5)

	1st Period	2nd Period	3rd Period
Norepinephrine loading study			
Fr hx	115.4 ± 33.1	106.5 ± 29.8	99.8 ± 43.0
Fr x	99.7 ± 13.2	79.5 ± 12.7*	78.9 ± 8.6*
Fr ua	9.2 ± 1.3	6.8 ± 1.3†	6.3 ± 2.2†
Fr ox	20.6 ± 3.1	17.1 ± 3.1	15.6 ± 2.8*
Ccr	104 ± 6	101 ± 6	101 ± 8
Control study			
Fr hx	104.8 ± 16.7	101.9 ± 21.7	95.4 ± 27.1
Fr x	100.0 ± 14.3	104.0 ± 17.1	109 ± 19.6
Fr ua	8.5 ± 9.5	8.8 ± 1.3	8.8 ± 1.1
Fr ox	19.3 ± 3.6	19.8 ± 3.2	19.9 ± 3.1
Ccr	109 ± 7	108 ± 6	109 ± 6

NOTE. Values are expressed as mean ± SD; 1st period, 2nd period, and 3rd period are described in Fig 1.

Abbreviations: Fr ua, percentage ratio of uric acid clearance/creatinine clearance (fractional uric acid clearance); Fr hx, percentage ratio of hypoxanthine clearance/creatinine clearance (fractional hypoxanthine clearance); Fr x, percentage ratio of xanthine clearance/creatinine clearance (fractional xanthine clearance); Fr ox, percentage ratio of oxypurinol clearance/creatinine clearance (fractional oxypurinol clearance).

*$P < .05$.

†$P < .01$.

creased by 1.13-fold ($P < .05$) and 1.19-fold ($P < .05$), respectively, in the second period and by 1.13-fold ($P < .05$) and 1.20-fold ($P < .05$), respectively, in the third period in the norepinephrine loading study (Table 4). On the other hand, these parameters did not change significantly in the control study (data not shown).

Effect of Norepinephrine Administration on the Urinary Excretion of Sodium, Chloride and Potassium

In the norepinephrine loading study, the urinary excretion of chloride and potassium decreased by 35% ($P < .05$) and 28% ($P < .05$), respectively, in the second period and by 38% ($P < .05$) and 48% ($P < .01$), respectively, in the third period (Table 5). The urinary excretion of sodium did not change significantly. On the other hand, these parameters did not change significantly in the control study (data not shown).

Table 4. Plasma Norepinephrine Concentration and Blood Pressure in the Norepinephrine Loading Study (N = 5)

	1st Period	2nd Period	3rd Period
Norepinrphrine (pg/mL)	263 ± 69	2,892 ± 410*	2,950 ± 423*
Systolic blood pressure (mm Hg)	105 ± 6	119 ± 7†	119 ± 8*
Diastolic blood pressure (mm Hg)	70 ± 8	83 ± 8†	84 ± 7†

NOTE. Values are expressed as mean ± SD; 1st period, 2nd period, and 3rd period are described in Fig 1.

*$P < .05$

†$P < .01$.

Table 5. Urinary Excretion of Sodium, Chloride, and Potassium in the Norepinephrine Loading Study (N = 5)

	1st Period	2nd Period	3rd Period
Na (μmol/h)	8.6 ± 3.8	5.5 ± 2.6	5.6 ± 3.4
Cl (μmol/h)	11.2 ± 2.9	7.3 ± 1.8*	6.9 ± 2.5*
K (μmol/h)	4.4 ± 1.1	3.1 ± 0.5*	2.3 ± 0.6†

NOTE. Values are expressed as mean ± SD; 1st period, 2nd period, and 3rd period is described in Fig 1.

*$P < .05$.

†$P < .01$.

Effect of Norepinephrine Administration on the Plasma Concentrations of Sodium, Chloride, and Potassium

In the norepinephrine loading study, the plasma concentrations of sodium, chloride, and potassium did not change significantly (Table 6), which was the same as for those in the control study (data not shown).

DISCUSSION

In the present study, it was demonstrated that the urinary excretion and fractional clearance of uric acid, xanthine, and oxypurinol (Tables 2 and 3) were decreased by norepinephrine, indicating that either the reabsorption of uric acid, xanthine, and oxypurinol was accelerated, or their secretion was inhibited by norepinephrine. Uric acid handling by the kidney is complex and confined to the proximal tubule. The renal transport of uric acid depends on both the urate/anion exchange pathway (reabsorption pathway) and the voltage sensitive pathway (secretion pathway).[10] The reabsorption pathway plays a major role in uric acid transport in humans, rats, and dogs, while the secretory pathway plays a major role in that pathway in pigs and rabbits, which have no brush border membrane anion exchange pathway.[10-15] Previous reports[2-6] have suggested that uric acid, xanthine, and oxypurinol share a renal transport pathway. In addition, a recent study[10] using human brush border membrane vesicles demonstrated that uric acid shares both a urate/anion exchanger and a voltage sensitive pathway with oxypurinol. Furthermore, a previous study[1] showed that norepinephrine decreased effective renal plasma flow and the urinary excretion of uric acid and potassium, but not the urinary excretion of sodium or creatinine clearance, suggesting that a reduction in effective renal plasma flow was related to a decrease in the urinary excretion of uric acid. In the present study, similar results were obtained for minerals and creatinine clearance, although effective renal plasma flow was not measured. Therefore, the present data for purine bases and oxypurinol (Tables 2 and 3) suggest that norepinephrine may affect a common

Table 6. Plasma Concentrations of Sodium, Chloride, and Potassium in the Norepinephrine Loading Study (N = 5)

	1st Period	2nd Period	3rd Period
Na (mEQ/L)	140 ± 1	140 ± 11	140 ± 1
Cl (mEQ/L)	103 ± 1	103 ± 1	102 ± 1
K (mEQ/L)	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1

NOTE. Values are expressed as mean ± SD; 1st period, 2nd period, and 3rd period are described in Fig 1.

renal transport pathway of uric acid, xanthine, and oxypurinol (the urate/anion exchange pathway and/or voltage sensitive pathway) via changes in hemodynamics, resulting in a decrease in the urinary excretion of uric acid, xanthine and oxypurinol. However, the possibility cannot be excluded that norepinephrine may directly affect this common renal transport pathway.

A previous epidemiologic study[16] showed an independent positive association between serum uric acid level and the development of hypertension. Furthermore, other studies[17,18] have demonstrated a decrease in the clearance of uric acid in patients with hypertension. Although the etiology of essential hypertension remains undetermined, many kinds of functional changes, such as those in the sympathetic nervous, renin-angiotensin-aldosterone, and kallikurein-kinin systems, are related to the development of hypertension. Therefore, the present findings suggest that high sympathetic tone may play a role, in part, in the decrease in the clearance of uric acid, as well as the development of hypertension. In addition, it is suggested that the hypouricemic effect of allopurinol may be more potent than that expected in gout patients with enhanced sympathetic tone, such as in salt-sensitive hypertension,[19-21] since the overall antihyperuricemic effect of allopourinol is mainly dependent on the plasma concentration of oxypurinol.

In a previous study,[22] it was shown that stimulation of sympathetic nerves around the portal vein and hepatic artery, as well as norepinephrine infusion, increased uric acid production in perfused rat livers, indicating that an exaggerated sympathetic tone can increase uric acid formation in the liver. However, because allopurinol was the administered agent in the present study, it is difficult to interpret from our plasma concentration of purine bases findings whether purine degradation in the liver was affected by norepinephrine. Nevertheless, in the preliminary data, without administration of allopurinol, the plasma concentration of purine bases was not affected by norepinephrine infusion (unpublished data), suggesting that purine degradation is not so affected in humans.

In conclusion, our data regarding to the effect of norepinephrine on the urinary excretion of purine bases and oxypurinol suggest that norepinephrine decreases the fractional clearance of uric acid, xanthine, and oxypurinol via hemodynamic change. In addition, it is suggested that the hypouricemic effect of allopurinol may be more potent than that expected in gout patients with enhanced sympathetic tone, such as in salt-sensitive hypertension. Further study is required to elucidate the exact mechanism that causes a decrease in the fractional clearance of uric acid and oxyourinol due to norepinephrine and the hypouricemic effect of allopurinol in gout patients with salt-sensitive hypertension.

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