Effect of Isoproterenol on Renal Uric Acid Excretion in Rats

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Abstract—Effect of isoproterenol on renal uric acid excretion was examined in oxonate-loaded and oxonate-nonloaded rats, using a clearance technique. Oxonate loading was performed by continuous infusion into the femoral vein. Isoproterenol (50 μ g/kg, i.v.) induced antidiuresis and hyperuricemia accompanied with decreases in urinary excretion rate of uric acid, uric acid clearance, inulin clearance, and uric acid clearance/inulin clearance ratio in oxonate-nonloaded rats. These effects of isoproterenol were inhibited by propranolol (1 mg/kg, i.v.). Very similar results were obtained in oxonate-loaded rats; i.e., systemic blood pressure decreased synchronously with uric acid excretion by isoproterenol. In contrast, phenylephrine (100 μ g/kg, i.v.) induced hypertensive, diuretic and slightly uricosuric results. In allopurinol-pretreated and oxonate-loaded rats, isoproterenol also decreased renal uric acid excretion and showed a less potent hyperuricemic effect than that observed in the animals not pretreated with allopurinol. In addition, in rats with ligated renal vessels, the hyperuricemic effect of isoproterenol was completely inhibited by allopurinol. These results suggest that isoproterenol-induced hyperuricemia is related not only to the stimulation of uric acid production, but also to the depression of renal uric acid excretion.

Catecholamines such as epinephrine and isoproterenol are known to induce hyperuricemia in rats (1, 2). Regulatory mechanisms of plasma uric acid level involve alterations of both uric acid production and uric acid excretion (3). Yonetani et al. (4) reported that hyperuricemia induced by epinephrine and isoproterenol in rats is caused by the stimulation of uric acid production. However, little information on the relationship between the hyperuricemic and renal actions of isoproterenol is available. Using a clearance technique in rats loaded with potassium oxonate, a uricase inhibitor (5), the present study was designed to determine whether or not the mechanism of hyperuricemic action of isoproterenol involves alteration of renal uric acid excretion.

Materials and Methods

Animals and treatments: Male Wistar rats, 7 weeks of age, were anesthetized with urethane (1.2 g/kg, s.c.). The left femoral vein was cannulated with a polyethylene

tube (PE-10) for infusion of loading solution. The loading solutions were as follows: Fluid I contained 4% mannitol, 1.5% inulin and 0.85% NaCl. Fluid II consisted of fluid I with 0.1% oxonate and 0.03% NaHCO₃. Fluid III consisted of fluid I with 1.5 mM uric acid, 0.1% oxonate and 0.055% NaHCO₃. Uric acid was added to obtain a higher plasma level. The loading solution was infused at a rate of 3.2 ml/hr. The urinary bladder was catheterized with Tygon tubing for collection of urine. After a 60 min equilibrium period, urine collection, blood collection and drug administration were done as follows: 10 min urine was collected continuously. Blood (0.37 ml, each) was taken from the jugular vein with a heparinized syringe 10 min before, and 10 and 30 min after the administration of test drugs or saline as control (Fig. 1). After centrifugation of the blood, the plasma sample was obtained.

Uric acid concentrations in plasma and urine were measured by the phosphotungstate colorimetric assay using a Uric Acid-Test

Fig. 1. Experimental protocol for collection of urine and blood and administration of drugs.

Wako kit (Wako). Inulin concentrations in plasma and urine were determined by the fluorometric method of Vurek and Pegram (6).

Systemic blood pressure was measured with a pressure transducer (Nihon Kohden, MPU-0.5) from a polyethylene tube (PE-50) inserted into the right femoral artery.

Rats with ligated renal vessels were prepared as follows: The arteries and veins of both kidneys were ligated at the neck of the kidney.

Chemicals: The following drugs were used: isoproterenol hydrochloride (Nikken Kagaku), phenylephrine hydrochloride (Tokyo Kasei), dl-propranolol hydrochloride and allopurinol (Sigma), mannitol and inulin (Nakarai), potassium oxonate (Calbiochem-Behring). Other chemicals were of reagent grade. Phenylephrine and propranolol were dissolved in saline. Isoproterenol was diluted with saline. Allopurinol was suspended in 0.2% sodium carboxymethyl cellulose.

Statistical analysis: The data are expressed as the mean $\pm$ S.D. and statistically analyzed by Student's *t*-test.

Results

Effect of isoproterenol on renal uric acid excretion in oxonate-nonloaded rats: Fluid I was used as the loading solution in this experiment.

Urine volume (U_{vol}), urinary excretion rate of uric acid (U_{UA}), plasma concentration of uric acid (P_{UA}), uric acid clearance (C_{UA}), inulin clearance (C_{In}) and uric acid clearance/inulin clearance ratio (C_{UA}/C_{In}) before drug administration were 26.5 ± 2.7 μ l/min, 4.35 ± 1.40 μ g/min, 0.55 ± 0.10 mg/dl, 0.769 ± 0.322 ml/min, 2.09 ± 0.46 ml/min and 0.380 ± 0.163 ,

respectively. In the control group, saline had no effect on any of the parameters. Isoproterenol (50 μ g/kg, i.v.) showed an antidiuretic effect immediately after the administration, and it also caused transient decreases in U_{UA} , C_{UA} , C_{In} and C_{UA}/C_{In} ; the decreases in U_{UA} , C_{UA} and C_{UA}/C_{In} were followed by increases. P_{UA} clearly increased (about 2-fold). Propranolol (1 mg/kg, i.v.) administered 1 min before isoproterenol completely blocked the effect of isoproterenol. Propranolol alone or propranolol plus isoproterenol induced mild diuresis (Fig. 2).

Effects of isoproterenol and phenylephrine on renal uric acid excretion and blood pressure in oxonate-loaded rats: Fluid II was infused in this experiment.

U_{vol} , U_{UA} , P_{UA} , C_{UA} , C_{In} and C_{UA}/C_{In} before drug administration were 26.9 ± 4.8 μ l/min, 31.7 ± 4.9 μ g/min, 1.95 ± 0.35 mg/dl, 1.61 ± 0.28 ml/min, 2.17 ± 0.39 ml/min and 0.784 ± 0.141 , respectively; oxonate produced higher levels of U_{UA} , P_{UA} , C_{UA} and C_{UA}/C_{In} than those obtained in oxonate-nonloaded rats, but it did not affect U_{vol} and C_{In} . The antidiuretic effect of isoproterenol (50 μ g/kg, i.v.) in oxonate-loaded rats was almost the same in potency as in oxonate-nonloaded rats. In addition, responses in the other parameters to isoproterenol in oxonate-loaded rats and oxonate-nonloaded rats were similar except that, in oxonate-loaded rats, increases in C_{UA} and C_{UA}/C_{In} at 30 min after the administration were not observed. In contrast, phenylephrine (100 μ g/kg, i.v.) was diuretic and transiently increased U_{UA} , but did not affect P_{UA} , C_{UA} , C_{In} and C_{UA}/C_{In} (Fig. 3).

The effects of isoproterenol and phenyl-

ephrine on systemic (mean) blood pressure were evaluated simultaneously with clearance studies. In the control group, blood pressure did not change during the experimental period. Isoproterenol, however, lowered blood

pressure and at the same time decreased U_{Vol} and U_{UA} . Phenylephrine raised blood pressure at the same time increasing U_{UA} (Table 1).

Effect of isoproterenol on renal uric acid excretion in allopurinol-pretreated and oxonate-loaded rats: The animals were pretreated with allopurinol (100 mg/kg, p.o.) 2.5 hr before isoproterenol administration,

Fig. 2. Effects of isoproterenol and propranolol on uric acid excretion in rats. Isoproterenol was administered at time 0. Propranolol was administered 1 min before isoproterenol. Results are expressed as % changes from the values before drug administration. Each point represents the mean $\pm$ S.D. (N=5). * $P < 0.05$, ** $P < 0.01$, significantly different from the control. + $P < 0.05$, ++ $P < 0.01$, significantly different from isoproterenol. $\circ$: control, $\bullet$: isoproterenol (50 μ g/kg, i.v.), Δ : propranolol (1 mg/kg, i.v.), $\blacktriangledown$: propranolol+isoproterenol.

Fig. 3. Effects of isoproterenol and phenylephrine on renal uric acid excretion in oxonate-loaded rats. Each drug was administered at time 0. Results are expressed as % changes from the values before drug administration. Each point represents the mean $\pm$ S.D. (N=5-6). * $P < 0.05$, ** $P < 0.01$, significantly different from the control. $\circ$: control, $\bullet$: isoproterenol (50 μ g/kg, i.v.), Δ : phenylephrine (100 μ g/kg, i.v.).

Table 1. Effects of isoproterenol and phenylephrine on mean blood pressure in oxonate-loaded rats

Drug	Dose (μ g/kg)	Blood pressure (mmHg)			
		-10 min	0 min	10 min	30 min
Control		87 $\pm$ 14	84 $\pm$ 18	83 $\pm$ 19	84 $\pm$ 15
Isoproterenol	50	90 $\pm$ 9	31 $\pm$ 3**	72 $\pm$ 5	88 $\pm$ 1
Phenylephrine	100	97 $\pm$ 6	153 $\pm$ 11**	95 $\pm$ 3	91 $\pm$ 10

Each drug was administered intravenously at time 0. Data represent the mean $\pm$ S.D. (N=4-6).

** $P < 0.01$, significantly different from the control.

then infused with fluid III.

U_{Vol} , U_{UA} , P_{UA} , C_{UA} , C_{In} and C_{UA}/C_{In} before isoproterenol were $32.4 \pm 9.3 \mu\text{l}/\text{min}$, $27.5 \pm 7.2 \mu\text{g}/\text{min}$, $1.53 \pm 0.36 \text{ mg}/\text{dl}$, $1.81 \pm 0.27 \text{ ml}/\text{min}$, $2.13 \pm 0.36 \text{ ml}/\text{min}$ and 0.847 ± 0.155 , respectively. In these allopurinol-pretreated and oxonate-loaded rats, isoproterenol ($50 \mu\text{g}/\text{kg}$, i.v.) induced antidiuresis and caused a decrease followed by an increase in U_{UA} , while the increase in U_{UA} was less than that observed in the animals not

pretreated with allopurinol. P_{UA} increased slightly and gradually; the increase was statistically significant ($P < 0.01$). C_{UA} , C_{In} and C_{UA}/C_{In} decreased (Fig. 4).

Effect of isoproterenol on plasma uric acid in rats with ligated renal vessels: Ligation of renal vessels was performed to eliminate the influence of renal functions. After this operation, P_{UA} gradually increased. Isoproterenol ($50 \mu\text{g}/\text{kg}$, i.v.) potentiated the increase in P_{UA} . However, the hyperuricemic effect of isoproterenol was lost in rats pretreated with allopurinol ($100 \text{ mg}/\text{kg}$, p.o.) (Table 2).

Discussion

Because most uric acid is rapidly converted into allantoin by uricase in rats, treatment with oxonate is a very useful method for studying the regulatory mechanisms of plasma uric acid level in rats. Yonetani and Iwaki (7) reported the usefulness of rats intraperitoneally treated with oxonate for evaluating renal effects of drugs. De Rougemont et al. (8) stated that the infusion of oxonate did not influence the water and urate transport. Accordingly, the present experiments were carried out employing a clearance technique using rats infused continuously with oxonate in order to examine the effect of isoproterenol on renal uric acid excretion.

Demartini (9) reported that epinephrine and norepinephrine decreased urine flow, C_{UA} and C_{UA}/C_{In} in humans. In our preliminary experiment, epinephrine had a tendency to decrease U_{Vol} and U_{UA} ; but norepinephrine was diuretic and slightly uricosuric in rats. The discrepancy between these observations is ascribed to variable

Fig. 4. Effect of isoproterenol on renal uric acid excretion in allopurinol-pretreated and oxonate-loaded rats. Isoproterenol was administered at time 0. Results are expressed as % changes from the values before drug administration. Each point represents the mean $\pm$ S.D. ($N=6$). * $P < 0.05$, ** $P < 0.01$, significantly different from the control. $\circ$: control, $\bullet$: isoproterenol ($50 \mu\text{g}/\text{kg}$, i.v.).

Table 2. Effect of isoproterenol on plasma uric acid in rats with ligated renal vessels

Pretreatment	Drug	N	Plasma uric acid (mg/dl)
None	Saline	4	1.86 ± 0.45
	Isoproterenol	4	$2.99 \pm 0.80^*$
Allopurinol	Saline	4	0.69 ± 0.15
	Isoproterenol	4	0.62 ± 0.09

Allopurinol ($100 \text{ mg}/\text{kg}$, p.o.) was treated 60 min before ligation of renal vessels. Saline or isoproterenol ($50 \mu\text{g}/\text{kg}$, i.v.) was administered 10 min after the ligation. Data represent the mean $\pm$ S.D. of the values at 30 min after drug administration. * $P < 0.05$, significantly different from the control (saline control).

effects of catecholamines depending on dosage and route of administration (10). Consequently, isoproterenol was expected to change renal uric acid excretion.

We observed that isoproterenol produced antidiuresis and hyperuricemia in oxonate-loaded rats. These data agreed with those in the previous reports showing that isoproterenol is antidiuretic (11, 12) and hyperuricemic (2,4). This drug first decreased, then increased, U_{UA} , C_{UA} and C_{UA}/C_{In} ; therefore, it seems likely that alteration of renal uric acid excretion plays an important role in regulating P_{UA} . Propranolol induced mild diuresis and completely antagonized both the hyperuricemic effect and the renal effect of isoproterenol, suggesting that the effects of isoproterenol are related to beta-adrenergic stimulation.

Although no increases in C_{UA} and C_{UA}/C_{In} at 30 min after isoproterenol administration in oxonate-loaded rats were observed, other responses to isoproterenol were very similar to those obtained in oxonate-nonloaded rats. The decreases in C_{In} shown in these two animal groups were less than those in C_{UA} , resulting in the decreases in C_{UA}/C_{In} . These results indicate that the inhibitory action of isoproterenol on renal uric acid excretion is due to both interference with glomerular filtration and alteration of tubular transport of uric acid. Isoproterenol increases sodium reabsorption, leading to a decrease in renal sodium excretion (13). The reabsorption of sodium parallels that of uric acid (14). Therefore, the decrease in C_{UA}/C_{In} observed in the present study may reflect an increase in reabsorption of uric acid. Further studies are required to clarify this point.

We examined phenylephrine in oxonate-loaded rats. As phenylephrine caused a transient increase in U_{UA} with diuresis, it is thought that if alpha-agonists such as phenylephrine and norepinephrine cause hyperuricemia (4, 15), the mechanism of the hyperuricemic action mediated by alpha-adrenoceptors may involve only the stimulation of uric acid production.

Renal functions are closely related to blood pressure (16), and hypotensive action of isoproterenol is caused by stimulation of beta₂-adrenoceptors (17, 18). In the present

study, the hypotensive effect of isoproterenol and the hypertensive effect (19) of phenylephrine were observed, and the changes in blood pressure were synchronized with those in U_{UA} . These data point out the relationship between blood pressure and renal uric acid excretion, and they support our previous study in which the hyperuricemic action of isoproterenol was suggested to be related to beta₂-adrenoceptors rather than to beta₁-adrenoceptors (15).

When the animals receiving the loading solution containing oxonate and uric acid were pretreated with allopurinol, isoproterenol caused mild hyperuricemia. This increase in P_{UA} was probably due to the accumulation of uric acid in plasma induced by the depression of renal uric acid excretion. In addition, in rats which had lost renal functions as a result of ligation of renal vessels, the hyperuricemic effect of isoproterenol was completely inhibited by allopurinol.

In conclusion, the mechanisms of hyperuricemic action of isoproterenol involve not only the stimulation of uric acid production, but also the depression of renal uric acid excretion.

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