CATECHOLAMINE-INDUCED HYPERURICEMIA IN EVISCERATED RATS WITH FUNCTIONAL HEPATECTOMY

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Abstract—The presence of extra-hepatic systems for uric acid production and their role in catecholamine-induced hyperuricemia were studied in eviscerated rats with functional hepatectomy. In these animals, the plasma uric acid level progressively increased with a decrease of allantoin, and isoproterenol subcutaneously administered in low doses produced an evident hyperuricemia. The effect of isoproterenol was seen even in nephrectomized animals, but all effects were abolished by pretreatment with allopurinol. Epinephrine and norepinephrine also produced hyperuricemia, though to a lesser extent than isoproterenol. Propranolol inhibited the hyperuricemic effects of isoproterenol and epinephrine, while phentolamine potentiated the effects of epinephrine. Electrical stimulation of the splanchnic nerve also produced hyperuricemia in eviscerated rats with functional hepatectomy, and this state was abolished by pretreatment with propranolol and by adrenalectomy but was not affected by pretreatment with phentolamine. Thus, beta adrenoceptor agonists stimulate production of uric acid in tissues other than the liver and the viscera, and hyperuricemia results. An effect similar to that on the uric acid level was also observed for the levels of plasma lactate, creatinine and glutamic oxalacetic transaminase in rats given isoproterenol.

We have already reported the hyperuricemic effects of catecholamine as characterized by inhibition of alpha and beta adrenoceptor antagonists (1, 2). These effects were due to enhancement of the production of uric acid, but the organs or tissues for its production were not determined. The liver and the intestines are generally thought to be main organs in the production of uric acid because of the high activity of xanthine oxidase, a rate-limiting enzyme for uric acid production (3). On the other hand, although the role of uric acid production in tissues other than the liver and intestines is presumed, experiments on the uric acid production have apparently not been carried out using eviscerated rats with or without functional livers, although

studies of various plasma components have been reported (4-6). In the present study, eviscerated rats with functional hepatectomy, prepared basically according to the method of Penhos et al. (5), readily produced uric acid without further metabolism to allantoin, these so-prepared rats proved to be good materials for evaluating drug effects on the production of uric acid in tissues other than the liver and the viscera. Beta adrenoceptor agonists could produce a marked hyperuricemia even in these animals.

MATERIALS AND METHODS

Drugs: Drugs administered were potassium oxonate (Calbiochem), allopurinol (Sigma), 1-epinephrine (Merck), 1-norepin-

ephrine (Sigma) and the following solutions for injection: 1-isoproterenol hydrochloride (Nikken Kagaku), dl-norepinephrine (Sankyo), propranolol hydrochloride (Sumitomo Kagaku) and phentolamine mesylate (CIBA).

Potassium oxonate and allopurinol were suspended in 1% gum arabic solution. 1-Epinephrine and 1-norepinephrine were dissolved with diluted hydrochloric acid and diluted with saline just before use.

Solutions for injection were diluted with saline, if necessary. All agents were usually administered in a volume of 2 ml/kg.

Animals and surgical procedures: Eight-week-old, male Wistar strain rats were used, except in the experiments with electrical stimulation in which 17-week-old rats were used. Evisceration with functional hepatectomy was done under anesthesia with sodium pentobarbital (50 mg/kg i.p.) basically according to the method of Penhos et al. (5).

In order to produce functional hepatectomy, the coeliac artery was tied and cut together with the anterior mesenteric artery, and the bile duct was ligated and cut with the portal vein. In this operation, the stomach, small and large intestines, pancreas, mesentery and spleen were excised. Nephrectomy or adrenalectomy was performed, if necessary.

Functionally hepatectomized rats without evisceration were prepared as follows: under anesthesia with sodium pentobarbital, the portal vein blood was transferred into the femoral vein at a flow rate of 3.0 ml/min by means of a peristaltic pump (Tokyo Rikakikai Co.). The operated animals were given 5 ml/kg of 5% glucose solution s.c. every hour, and nephrectomized or adrenalectomized animals were given 5 ml/kg of saline s.c. in addition to the glucose solution.

Methods for drug administration and blood collection: In eviscerated rats with functional hepatectomy and animals only with functional

hepatectomy, all drugs were administered subcutaneously immediately after the operation, except allopurinol which was given p.o. one hour before the operation. Animals of the control group received saline instead of drug solution, and blood was collected from the jugular vein repeatedly, except in the experiments for estimating plasma components other than purine catabolites in which blood was collected from the aorta at one hour after drug administration. Potassium oxonate was administered to intact rats in order to inhibit the urate oxidase, according to Johnson et al. (7). This agent was given at 250 mg/kg i.p. under anesthesia with sodium pentobarbital, while isoproterenol was administered i.p. at 30 min after the treatment with potassium oxonate. Blood samples were collected from the jugular vein.

Electrical stimulation of the splanchnic nerve: The left splanchnic nerve of eviscerated rats with functional hepatectomy was exposed immediately after the elimination of viscera, and mounted on a bipolar electrode, then the abdominal incision was closed except for the site around the electrode after the exposed nerve had been covered with liquid paraffin. In these animals, adrenoceptor antagonists were administered prior to exposure of the splanchnic nerve. Blood was collected first from the jugular vein just before the electrical stimulation was given and again one hour later. The stimulation with 10-sec rectangular pulses (1 msec, 5 V, 20 c/sec) was given fifteen times at 10-sec intervals, and the stimulation was repeated four times every 15 min. The animals of the control group were given all treatments except the electrical stimulation.

Preparation of tissue extracts and determination of enzyme activities: Tissues were excised immediately after sacrifice by decapitation, then homogenized with nine volumes of 0.05 M phosphate buffer, pH 8.0.

The homogenate was partly used for the

determination of urate oxidase activity, and the residue was given the following treatments in order to remove the inhibitors in the determination of xanthine oxidase activity. The homogenate was centrifuged for 10 min at $25,000\times g$ and recentrifuged after adjustment to pH 5.0 with 5 N acetic acid. The supernatant fluid was neutralized with 1 N sodium hydroxide, then the volume was adjusted. A 2-ml portion of the solution was gel-filtrated on a column packed with 15 ml of Sephadex G-25, which was eluted with water. Ten milliliters of eluent was used as the material for the determination of xanthine oxidase activity.

The activities of urate oxidase and xanthine oxidase were estimated basically according to Kalckar's method (8). The quantitative accuracy of the assay methods, shown in Fig. 1, was determined using enzyme materials extracted from the liver.

To determine the urate oxidase activity, 1 ml of 0.1 M borate buffer, pH 9.0, was mixed with 0.5 ml of diluted homogenate, and the mixture was preincubated for 10 min at 37°C . Next, 0.5 ml of 5×10^{-4} M uric acid solution was added, then the mixture was incubated at 37°C for 10 min. The incubated mixture was mixed with 1.0 ml of 3 N hydrochloric acid, then centrifuged for 10 min at 3,000 r.p.m. The enzyme activity was assessed from the decrease in the optical density at 290 m μ in the supernatant fluid, and one unit was defined as the enzyme amount which oxidized 1×10^{-6} M uric acid per min.

Xanthine oxidase activity was determined using a 0.05 M phosphate buffer, pH 8.0, the eluent from Sephadex G-25 column as the enzyme material and 5×10^{-4} M xanthine solution as the substrate. The determination was performed in a manner similar to that for urate oxidase, except for incubation at 37°C for 30 min, and the activity was assessed from the increase of optical density at 290 m μ

Fig. 1. Time courses and relationships between enzyme amount and the activity in the procedures for determining urate oxidase and xanthine oxidase activities. Enzyme materials were prepared from rat liver. Panel A is the time course of urate oxidase activity, in which two concentrations of enzyme material ($[E]=2$ and 4 correspond to 2 and 4 of the relative amount of enzyme in panel B, respectively) were used. Panel B is the relationship between the amount of urate oxidase and the activity with incubation for 10 min at 37°C . Panel C is the time course of xanthine oxidase activity, while panel D is the relationship between the amount of xanthine oxidase and the activity with incubation for 30 min at 37°C .

due to the uric acid produced from xanthine. The enzyme activity was defined as one unit was the amount of enzyme which produced 1×10^{-6} M uric acid per min. The protein concentration in the tissue extracts was assessed with Bio-Rad reagent for protein assay.

Determination of plasma components: Blood was cooled in ice water after collection,

then the plasma was separated as soon as possible.

Uric acid and allantoin were determined by methods described previously (9). Lactate was estimated by Hohorst's method (10), and other plasma components were determined with an autoanalyzer (TECHNICON autoanalyzer, SMA-MICRO system), in which creatinine and glutamic oxalacetic transaminase were estimated by the method of Chasson et al. (11) and Kessler et al. (12), respectively.

RESULTS

The presence of extra-hepatic systems to produce uric acid in rats was confirmed from two points of view. The first was a study on the distribution of xanthine oxidase and urate oxidase in various tissues. Their activities in all tested materials were assessed as for those in the liver extracts shown in Fig. 1. As shown in Table 1, xanthine oxidase activity was found at different levels in various tissues, and particularly at a high level in the liver and the jejunum, whereas urate oxidase activity was recognized only in the liver and was not detectable elsewhere.

The second point of view was confirmed in a study using animals which had undergone functional hepatectomy, with or without

evisceration. As seen in Fig. 2, potassium oxonate-treated rats increased the plasma uric acid with a decrease of allantoin. Similar changes of uric acid and allantoin occurred in eviscerated rats with functional hepatectomy, though the allantoin level tended to increase at 3 hr after the operation. On the other hand, a functional hepatectomy in which the portal vein blood was transferred into the

Fig. 2. Effects of potassium oxonate (A), evisceration with functional hepatectomy (B), and functional hepatectomy without evisceration (C) on plasma uric acid and allantoin in rats. Experiments were done as described in the text. The filled circles show the mean values of plasma uric acid, while the open ones are those for allantoin. Bars give the standard errors. Each experiment was done with 6 animals. *, **: Significantly different from the initial level at $P < 0.05$ and 0.01 , respectively.

Table 1. Distributions of xanthine oxidase and urate oxidase activities in rat tissues

Tissue	Xanthine oxidase (units/mg protein)	Urate oxidase (units/mg protein)
Liver	1.41±0.05	4.14±0.25
Jejunum	3.86±0.84	n.d.
Spleen	1.00±0.01	n.d.
Kidney	0.84±0.05	n.d.
Lung	0.67±0.05	n.d.
Heart	0.44±0.01	n.d.
Thymus	0.42±0.02	n.d.
Testis	0.40±0.03	n.d.
Biceps femoris	0.23±0.01	n.d.
Brain	0.13±0.01	n.d.

Values are means±standard errors of 5 animals. n.d.: not detectable

femoral vein with a peristaltic pump markedly increased the plasma uric acid level with a gradual increase of allantoin.

Next, studies were done to evaluate the hyperuricemic effect of catecholamine in eviscerated rats with functional hepatectomy. Subcutaneously administered isoproterenol at a low dose markedly increased the plasma uric acid as shown in Fig. 3.

Similar progressive curves of isoproterenol-induced hyperuricemia were observed even when the agent at higher doses was administered i.p. to potassium oxonate-treated rats and s.c. to animals with functional hepatectomy. The increments of uric acid and allantoin during one hour after the administration of isoproterenol were compared among the three groups of animals (Fig. 4). The increment of uric acid reached maximum in each group when the doses of isoproterenol were increased, and the level found in potassium oxonate-treated rats was the highest. The level in eviscerated rats with functional hepatectomy was also appreciable

Fig. 3. Effect of isoproterenol on plasma uric acid and allantoin in eviscerated rats with functional hepatectomy. 1-Isoproterenol hydrochloride (5 μ g/kg s.c.) was administered immediately after the operation. Values are means $\pm$ standard errors of 6 animals.

Fig. 4. Effect of isoproterenol on plasma uric acid and allantoin in potassium oxonate-treated rats (A), eviscerated rats with functional hepatectomy (B), and functionally hepatectomized rats without evisceration (C). The open columns show the mean increment of uric acid at one hour after the administration of isoproterenol, while the filled ones are those for allantoin. Values are means $\pm$ standard errors, and the number of animals is given in parentheses. *, **: Significantly different from the increment of the control group at $P < 0.05$ and 0.01 , respectively.

and more than half of the amount shown in potassium oxonate-treated rats. The animals with functional hepatectomy and artificial circulation of portal vein blood showed the most notable increase of plasma uric acid in the operation, but a less increment of uric acid than that in eviscerated rats with functional hepatectomy upon administration of isoproterenol. On the other hand, the allantoin levels in potassium oxonate-treated rats and eviscerated rats with functional hepatectomy had no appreciable change upon administration of isoproterenol, but the level in rats with functional hepatectomy slightly increased.

The plasma volumes in intact rats and eviscerated rats with functional hepatectomy were assessed under anesthesia with sodium pentobarbital according to Gregersen's

method (13). The volume was 8.2 ± 0.1 ml (mean $\pm$ standard error, $n=6$) in intact rats and 5.5 ± 0.3 ml ($n=6$) in eviscerated rats with functional hepatectomy.

The characteristics of catecholamine-induced hyperuricemia in eviscerated rats with functional hepatectomy were studied in detail.

In the following experiments, the effects of administered drugs were evaluated by the increment of plasma uric acid during one hour after the administration. Epinephrine and norepinephrine also induced hyperuricemia in the animals (Table 2), but epinephrine was less active than isoproterenol, and norepinephrine was the least effective.

As indicated in Table 3, nephrectomy increased the plasma uric acid level in eviscerated rats with functional hepatectomy,

Table 2. Hyperuricemic effects of catecholamine in eviscerated rats with functional hepatectomy

Treatment	n	Increment of plasma uric acid (mg/dl)
Control	7	1.29 ± 0.07
1-Isoproterenol-HCl, 50 μ g/kg, s.c.	6	$9.49 \pm 0.74^{**}$
1-Epinephrine, 50 μ g/kg s.c.	6	$3.66 \pm 0.48^{**}$
1-Norepinephrine, 50 μ g/kg s.c.	6	1.48 ± 0.12
dl-Norepinephrine, 500 μ g/kg s.c.	6	$2.14 \pm 0.14^{**}$

Drugs were administered as described in the text, and the increment of plasma uric acid during one hour after the administration is shown as the mean $\pm$ standard error. **: Significantly different from the control value at $P < 0.01$.

Table 3. Effect of nephrectomy on the hyperuricemic effect of isoproterenol in eviscerated rats with functional hepatectomy

Treatment	n	Increment of plasma uric acid (mg/dl)
Evisceration with functional hepatectomy (A)	6	1.28 ± 0.09
Evisceration with functional hepatectomy and nephrectomy (B)	6	$2.21 \pm 0.17^{**}$
(B) + 1-Isoproterenol-HCl, 5 μ g/kg s.c.	6	$6.87 \pm 0.38^{††}$

The animals in groups A and B were administered saline, and the increment of plasma uric acid was estimated one hour later as described in the text. Data represent means $\pm$ standard errors.

** : Significantly different from (A) at $P < 0.01$. †† : Significantly different from (B) at $P < 0.01$.

but isoproterenol also caused hyperuricemia in the animals which had received evisceration with functional hepatectomy and nephrectomy.

On the other hand, as seen in Table 4, the pretreatment with allopurinol and the concomitant administration of propranolol obviously abolished the hyperuricemic effect of isoproterenol. The hyperuricemic effect of epinephrine was also abolished more ap-

parently by the concomitant administration of propranolol, and potentiated by phentolamine.

Next, the hyperuricemic effect in the electrical stimulation of the splanchnic nerve in eviscerated rats with functional hepatectomy was studied in relation to the action of endogenous catecholamine. The stimulation, as shown in Table 5, produced hyperuricemia, which was obviously

Table 4. Effects of allopurinol and adrenoceptor antagonists on catecholamine-induced hyperuricemia in eviscerated rats with functional hepatectomy

Treatment	n	Increment of plasma uric acid (mg/dl)
Control	6	1.22±0.11
1-Isoproterenol·HCl, 50 µg/kg s.c.	8	7.89±0.31**
Allopurinol, 100 mg/kg p.o.	6	0.16±0.01**
Allopurinol, 100 mg/kg p.o. & 1-Isoproterenol·HCl, 50 µg/kg s.c.	9	0.28±0.04**
Propranolol·HCl, 0.5 mg/kg s.c.	6	1.08±0.13
Propranolol·HCl, 0.5 mg/kg s.c. & 1-Isoproterenol·HCl, 50 µg/kg s.c.	6	1.61±0.09*
Control	6	1.22±0.04
1-Epinephrine, 100 µg/kg s.c.	6	4.28±0.45**
Propranolol·HCl, 0.5 mg/kg s.c. & 1-Epinephrine, 100 µg/kg s.c.	6	0.62±0.08**
Phentolamine mesylate, 1.0 mg/kg s.c.	6	1.25±0.22
Phentolamine mesylate, 1.0 mg/kg s.c. & 1-Epinephrine, 100 µg/kg s.c.	6	7.67±0.38**

Drugs were administered as described in the text. The increment of plasma uric acid during one hour after the administration of catecholamine was studied. Values represent means ±standard errors. *, **: Significantly different from the control value at $P<0.05$ and 0.01 , respectively.

Table 5. Effect of electrical stimulation of the splanchnic nerve on plasma uric acid levels in eviscerated rats with functional hepatectomy

Pretreatment	Increment of plasma uric acid (mg/dl)	
	Without stimulation	With stimulation
None	0.97±0.09 (6)	2.45±0.30** (6)
Propranolol·HCl, 1 mg/kg s.c.	1.09±0.16 (6)	1.31±0.15 (6)
Phentolamine mesylate, 2 mg/kg s.c.	0.77±0.09 (7)	2.54±0.17** (7)
Adrenalectomy	0.99±0.07 (6)	1.30±0.12 (7)

Experiments were carried out as described in the text. The increment of plasma uric acid during one hour after the start of electrical stimulation was studied. Values represent means ±standard errors, and the number of animals is given in parentheses. **: Significantly different from the value of the group without stimulation at $P<0.01$.

Table 6. Effect of isoproterenol on plasma uric acid, lactate, creatinine and glutamic oxalacetic transaminase (GOT) in eviscerated rats with functional hepatectomy

Treatment	Plasma components			
	Uric acid (mg/dl)	Lactate (μ moles/ml)	Creatinine (mg/dl)	GOT (mU/ml)
Control	1.68 $\pm$ 0.11	2.65 $\pm$ 0.52	0.5 $\pm$ 0.0	144 $\pm$ 3
1-Isoproterenol-HCl, 10 μ g/kg s.c.	9.87 $\pm$ 0.53**	9.58 $\pm$ 0.53**	0.7 $\pm$ 0.0**	164 $\pm$ 7*
Propranolol-HCl, 0.5 mg/kg s.c. & 1-Isoproterenol-HCl, 10 μ g/kg s.c.	2.01 $\pm$ 0.10††	4.11 $\pm$ 0.50††	0.5 $\pm$ 0.0††	136 $\pm$ 7†

Blood was collected from the aorta at one hour after drug administration as described in the text. Values represent means $\pm$ standard errors of 8 animals. *, **: Significantly different from control group at $P<0.05$ and 0.01 , respectively. †, ††: Significantly different from isoproterenol-treated group at $P<0.05$ and 0.01 , respectively.

abolished by pretreatment with propranolol and by adrenalectomy, but not affected by phentolamine.

The effects of evisceration with functional hepatectomy and administration of isoproterenol to the operated animals were also studied for various plasma components other than purine catabolites.

Increases due to the operation were also observed in the concentrations of urea, bilirubin, chloride and cholesterol, and the activities of glutamic oxalacetic transaminase and alkaline phosphatase, though these changes were much less than that of uric acid. On the other hand, isoproterenol also caused changes of lactate, creatinine and glutamic oxalacetic transaminase like that of uric acid, as shown in Table 6.

DISCUSSION

Because of the abundant content of xanthine oxidase and other purine-metabolizing enzymes, mammalian liver is generally thought to be an important organ in which uric acid is produced. However, the liver, except for that in primates, is also the organ for further metabolism of uric acid to allantoin by urate oxidase. An earlier study suggested the presence of urate oxidase even in the kidneys and the spleen (14), but we did not detect this activity in tissue extracts other

than the liver. On the other hand, xanthine oxidase activity was recognized in various tissues, although it could not be distinguished from that of aldehyde oxidase because of interference from crude materials. Thus, the presence of an enzyme which can catalyze the metabolism of xanthine to uric acid supports the possibility of production of uric acid in the tissue. The increase of plasma uric acid level without a similar change of allantoin after functional hepatectomy with or without evisceration also supported the presence of extra-hepatic systems for uric acid production. However, a slight increase in plasma allantoin levels in eviscerated rats with functional hepatectomy and its more apparent increase in functionally hepatectomized animals without evisceration (Fig. 2) may be due to the presence of urate oxidase in tissues other than the liver or renal disturbance in the operated animals. The degree of increase of plasma uric acid due to evisceration with functional hepatectomy was similar to that in potassium oxonate-treated rats, while that in functionally hepatectomized rats without evisceration was much greater. These results indicate the importance of extra-hepatic systems for uric acid production, but cannot explain the rates of the uric acid production in the liver, the viscera and other tissues, because of various problems based upon

experimental conditions in each group of animals. On the other hand, the hyperuricemic effect of isoproterenol (Fig. 4) indicated the importance of the extra-hepatic systems in addition to the hepatic system for uric acid production. A higher increment of plasma uric acid resulting from the administration of isoproterenol in potassium oxonate-treated rats than that in animals with functional hepatectomy suggests that the liver may be important for the uric acid production elicited by isoproterenol. However, an appreciable increment of plasma uric acid in eviscerated rats with functional hepatectomy indicated the importance of tissues other than the liver and the viscera in isoproterenol-induced hyperuricemia.

On the other hand, functionally hepatectomized rats without evisceration showed an isoproterenol-induced increment of plasma uric acid less than that in eviscerated rats with functional hepatectomy in spite of the notable increase of plasma uric acid due to the operation. Accordingly, the uric acid production in tissues other than the liver and the viscera is presumed to be important for uric acid production elicited by isoproterenol in extra-hepatic systems.

The hyperuricemic effect of isoproterenol in eviscerated rats with functional hepatectomy and nephrectomy, and the effect of allopurinol on isoproterenol-induced hyperuricemia supported the occurrence of a stimulated production of uric acid with administration of isoproterenol.

The different efficiencies among isoproterenol, epinephrine and norepinephrine, and the effect of propranolol characterized the hyperuricemic effect of catecholamine in eviscerated rats with functional hepatectomy as being due to the action of the beta adrenoceptor agonist. The hyperuricemia induced by electrical stimulation of the splanchnic nerve in the operated rats was also blocked by pretreatment with propranolol.

Sumi and Umeda (15, 16) already reported a hyperuricemia by electrical stimulation of the ventromedial hypothalamus in rats. The hyperuricemia was abolished by adrenalectomy and by treatment with propranolol but greatly potentiated by phentolamine. In our experiments, adrenalectomy had no effect on the increase of plasma uric acid in eviscerated rats with functional hepatectomy, but obviously abolished the hyperuricemia induced by electrical stimulation of the splanchnic nerve. Phentolamine potentiated the hyperuricemic effect of exogenously administered epinephrine but had no effect in the electrical stimulation of the splanchnic nerve. Thus, though the effects of phentolamine were not always similar, adrenal epinephrine released by nerve stimulation must have produced hyperuricemia due to the action on the beta adrenergic receptor. Moreover, the present study demonstrated that such a hyperuricemia due to the action of endogenous catecholamine occurred even in eviscerated rats with functional hepatectomy.

The present study showed the appreciable function of extra-hepatic systems in uric acid production in rats. Das and Steinberg (17) recently reported a role of the lung in the hyperuricemic effect of fructose. The effect of isoproterenol in our experiments on plasma levels of lactate, creatinine and glutamic oxalacetic transaminase similar to that on uric acid in eviscerated rats with functional hepatectomy suggests that the hyperuricemic effect of isoproterenol may be related to its stimulation of energy metabolism in the myocardium or other muscles. Accordingly, the uric acid production in the cardiopulmonary systems or in the skeletal muscle should be studied to fully understand the mechanisms of catecholamine-induced hyperuricemia. Another important problem is whether the presence of extra-hepatic systems for uric acid production as mentioned

above is specific in animals such as rats, and hence the hyperuricemic effect of catecholamine should be investigated even in animals such as chickens with functional hepatectomy.

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