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Origin of Plasma Uric Acid induced by *l*-Epinephrine[1)]

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The origin of increased plasma uric acid in *l*-epinephrine-induced hyperuricemia was studied. It was shown that uric acid is increased by an extra-hepatic process, in particular by reduction in the intestinal adenosine-tri-phosphate level.

Further, from evidence for the existence of plasma xanthine oxidase, it was shown that production of uric acid by an extra-hepatic process is very likely in many mammals, even in man.

Keywords——uric acid; ATP; *l*-epinephrine; xanthine oxidase; hyperuricemia; phenoxybenzamine; liver; intestine; plasma; rat

It is generally accepted that most of the uric acid produced in mammals is formed in the liver through the *de novo* biosynthesis of purine nucleotides and their catabolism to uric acid.[3)] Accordingly, it has been assumed that the hyperuricemic state is induced either by the over production of purine compounds, elicited to remove feedback control on biosynthesis, or by impaired clearance in the kidney.

Recently, we presented a new consideration that the hyperuricemia induced by administration of catecholamines to experimental animals arises through a different mechanism, and moreover we gave evidence for the presence of a pathway producing uric acid in blood.[4,5)] In those reports, we made the important suggestion that the rapid elevation of plasma uric acid after administration of catecholamines is not dependent upon over-production of purine compounds in the liver. Rather, we considered it more likely that the mechanism is related to the degradation of membrane adenosine-tri-phosphate, which we postulated in relation to the action of catecholamines on their receptors.

In this report, we give further experimental evidence that hyperuricemia after *l*-epinephrine treatment is, in part, induced by the extra-hepatic formation of uric acid.

Materials and Methods

Experimental Animals——Male, seven-week old Wistar rats were used for most of the experiments. For the investigation of species difference, blood and plasma were obtained in addition from male five-week DS mice, male Hartley strain guinea pigs weighing 300 g, adult male beagle dog weighing about 8 kg, and normal men.

Determination of Tissue Adenosine-tri-phosphate (ATP)——Within a minute after decapitation, tissues were weighed and homogenized in 5% ice-cold perchloric acid. The concentration of homogenates was varied from 2 to 10% in respective tissues according to their ATP contents. Each homogenate was centrifuged in the cold for 15 minutes at 3000 rpm or 15000 rpm, then the resultant supernatant was pipetted in another tube and recentrifuged for 10 minutes after neutralization with 5 N potassium carbonate solution. The

1) This work was presented at the 74th Annual Meeting of Biochemical Society of Japan, Okayama, October 1974.
2) Location: *Fukushima-ku, Osaka, 553, Japan.*
3) S. Morisawa and M. Yukioka, *Metabolism and Disease*, **5**, 168 (1968).
4) Y. Yonetani, T. Douzaki, and Y. Ogawa, *Chem. Pharm. Bull.* (Tokyo), **25**, 441 (1977).
5) Y. Yonetani and Y. Ogawa, *Chem. Pharm. Bull.* (Tokyo), **25**, 448 (1977).

concentration of ATP in the supernatant was determined by a modification of the fluorophotometric procedure for determination of glucose using the hexokinase-glucose-6-phosphate dehydrogenase system.[6]

An aliquot (0.2 ml) of supernatant fluid was pipetted into a test tube and mixed with 0.1 ml of reagent solution containing 0.8 volume of 0.5 M Tris-hydrochloric acid buffer with 2 mM of magnesium chloride (pH 8.0), 0.1 volume of 0.012 M nicotinamide adenine dinucleotide phosphate, 0.1 volume of 0.03 M glucose, 0.005 volume of 0.2—0.5% hexokinase solution, and 0.01 volume of 0.1—0.5% glucose-6-phosphate dehydrogenase solution. After 20 minutes at room temperature, 2 ml of 0.01 N sodium hydroxide solution containing 1 mM of ethylenediamine-tetra-acetic acid disodium salt was added and the amount of reduced nicotinamide adenine dinucleotide phosphate was determined fluorophotometrically at 458 nm and 340 nm for emission wave length and excitation one, respectively. In the blank test, glucose and hexokinase were eliminated from the above reagent solution.

Determination of Plasma Xanthine Oxidase Activity——Five ml of plasma was mixed with 1 ml of 3×10^{-3} M xanthine solution (pH 7.0), and incubated at 37°. One ml of the reaction mixture was pipetted out after 0, 15-, 30-, and 60- minutes incubation, and mixed with 1.5 ml of 2% sodium tungstate and 0.6 ml of 0.33 N sulfuric acid. After centrifugation for 10 minutes at 3000 rpm, 2.0 ml of the supernatant fluid was mixed with 0.2 ml of a 30% sodium carbonate–45% urea (2: 1) mixture and 0.1 ml of Folin's phosphotungstate reagent.[7] The optical density of the mixture was determined at 710 nm after it had been left to stand for 25 minutes at room temperature and a unit of enzyme activity was defined as the amount which produced 0.01 μmole of uric acid per minute under the assay procedure.

Determination of Plassma Uric Acid——In the materials in which xanthine oxidase activity was determined, the concentration of plasma uric acid was calculated from the optical density at zero time. In other experiments, plasma uric acid was determined with autoanalyzer.[8]

Results

Elevation of Plasma Uric Acid by *l*-Epinephrine

The determined concentration of a plasma component varies depending on what part of the body the blood sample was taken from. From this variation we can get a rough picture of the metabolic fate of the plasma component. As seen in Fig. 1, the highest concentration of plasma uric acid in pentobarbital anesthetized rats was found in venous blood taken from the hepatic vein. Though a similar concentration was found in samples of arterial blood, venous blood taken from other locations had markedly lower concentration. The results in this figure correspond well with the generally accepted view that uric acid is formed in the liver and cleared through the kidney. The similar observations have been made on blood glucose, which is well known to be produced in the liver.

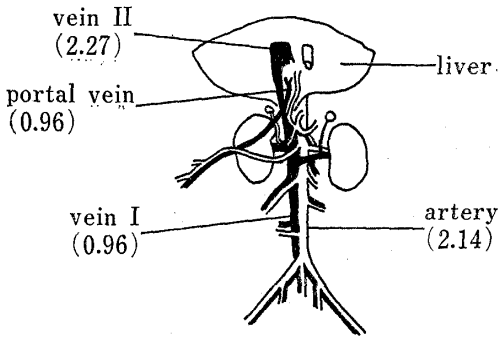

Fig. 1. Plasma Uric Acid in Rats

( ) uric acid, mg/dl
Each value shows average of two samples, each of which was collected from 4—6 rats.

Table I. Effect of *l*-Epinephrine on Plasma Uric Acid in Rats

Blood vessel	Time after injection (min)		
	0	5	15
	Uric acid, mg/dl		
Vein, II	2.27	1.62	3.05
Artery	2.14	1.90	3.44
Vein, I	0.96	1.05	2.59
Portal vein	0.96	1.11	4.22

l-epinephrine, 1.0 mg/kg, intraperitoneally
Each value shows average of two samples, each of which was collected from 4—6 rats.

6) P. Greengard, "Methods of Enzyme Analysis," ed. by H.U. Bergmeyer, Academic Press, New York and London, 1963, p. 537.
7) O. Folin and V. Ciocalteu, *J. Biol. Chem.*, 73, 627 (1927).
8) R.M. Archibalt, *Clin. Chem.*, 3, 102 (1957).

The differentiation in plasma uric acid concentration among blood vessels was remarkably modified under the administration of *l*-epinephrine, as shown in Table I. Epinephrine treatment also caused an increase in blood glucose concentration in all samples, but without any modification in the ranking of the concentrations.

These results show that plasma uric acid can be elevated by being formed at sites other than the liver. At 5 minutes after epinephrine administration, there was a slight reduction of uric acid concentration in vein II containing hepatic blood and in the artery in spite of a slight increase in the other veins, and 15 minutes after injection the uric acid concentration was markedly increased in all vessels, the concentration in the portal vein now being elevated to a higher level than that in vein II. It seems likely that initially after epinephrine treatment the liver has a slightly reduced ability to release uric acid, and that there is then a further modification, the main function changing from uric acid production to uric acid digestion.

On the other hand, the difference in uric acid concentration between artery and vein I was reduced by about 28% during the initial five minutes, and maintained at this level fifteen minutes after injection. Thus, it is impossible for the high level of uric acid in the portal vein to be attributed to disturbance in kidney function.

Correlation between Reduction of Tissue ATP Levels and Elevation of Plasma Uric Acid

As documented previously,[4] the induction of hyperuricemia by catecholamines is inhibited by pretreatment with α-blocking agents and ATPase inhibitors. Consequently, an understanding of the relationship between the reduction of tissue ATP levels and elevation of plasma uric acid should throw light on the source of uric acid elicited by epinephrine. As shown in Fig. 2, a marked reduction in liver ATP level accompanied the elevation of plasma uric acid after *l*-epinephrine injection in rats, but this reduction was inhibited by pretreatment with phenoxybenzamine, as was the elevation of plasma uric acid.

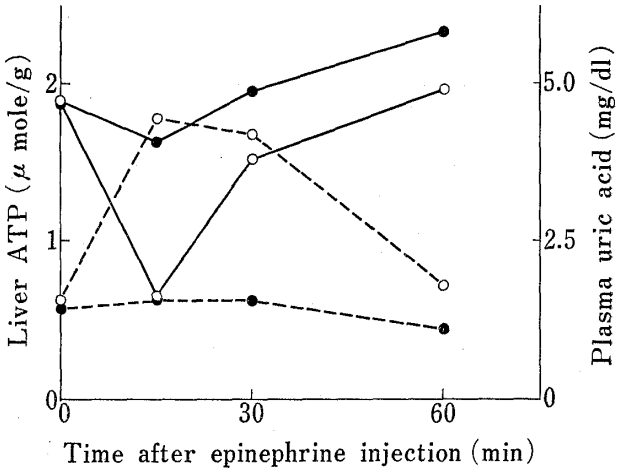

Fig. 2. Effects of *l*-Epinephrine on Liver ATP and Plasma Uric Acid in Rats

○: *l*-epinephrine, 1.0 mg/kg, administered intraperitoneally
●: phenoxybenzamine, 1.0 mg/kg, administered intraperitoneally 30 min before epinephrine.
——: liver ATP, ------: plasma uric acid
Each Point shows average value of 5 rats.

However, the reduction of liver ATP did not always follow the elevation of plasma uric acid. When *l*-epinephrine was injected at a lower dose, 0.1 mg/kg for example, the reduction

Table II. Effect of *l*-Epinephrine on Tissue ATP in Rats

Tissue	Exp. No.	Control	Reduction rate of ATP by epinephrine %
		μ moles/g. wet wt.	
Liver	3	1.49—1.89	60—65
Intestine	2	1.31, 1.76	48, 41
Kidney	2	1.16, 1.59	18, 26
Heart	3	2.26—3.35	2—20
Skeletal muscle	4	6.37—7.66	0—26

Value in each experiment shows average of 5—6 rats.
Tissues were removed 15 min after intraperitoneal administration of *l*-epinephrine, 1.0 mg/kg.

in liver ATP level was as big as that caused by a dose of 1.0 mg/kg and there was a clear increase in blood glucose, but there was no elevation of plasma uric acid. On the other hand, rapid reduction of ATP level was also observed in the intestine when 1.0 mg/kg of *l*-epinephrine was administered in rats, but only small reduction were seen in the kidney, heart, and skeletal muscle under the same epinephrine treatment, as listed in Table II. Reduction of intestinal ATP, corresponding to the elevation of plasma uric acid, was seen only after treatment with a high dose of epinephrine. An inhibitory effect of phenoxybenzamine was also seen in the intestine, though clear inhibition was not seen in the other tissues.

The minced intestine produced uric acid during the incubation at 30° in the medium of Krebs-Ringer Bicarbonate buffer, and 10^{-4} M of *l*-epinephrine enhanced the reaction in the earlier state both on the reduction of intestinal ATP and the production of uric acid in medium (Table III). From these results, it is reasonable to conclude that the intestine is at least one of the sources of plasma uric acid outside of the liver and is responsible for the remarkable elevation of uric acid in the portal vein indicated in Table I. Furthermore, it is thought that

Table III. Effect of *l*-Epinephrine on ATP Content and Uric Acid Production in Minced Intestine of Rat

Item	Incubation min	Control	*l*-Epinephrine
		μ moles	
ATP (Decrease in minced intestine)	0.5	0.11±0.01	0.20±0.03[a]
	1.0	0.27±0.02	0.32±0.05
	5.0	0.31±0.04	0.34±0.03
Uric acid (In medium)	5.0	0.14±0.01	0.29±0.03[b]

M ± s.e., $n=5$, *a*) $p<0.05$, *b*) $p<0.01$
Minced intestine (0.5 g) was incubated at 30° in the medium of KRB 2.0 ml and water or 6×10^{-4} M *l*-epinephrine solution 0.5 ml.

the *in vivo* formation of plasma uric acid may take place in the plasma, using the intermediates of ATP degradation from the intestine or other tissues, because the uric acid formation in minced intestine is far greater in plasma medium than in Krebs-Ringer Bicarbonate buffer under experimental condition in which there was no destruction of the minced intestine during incubation.

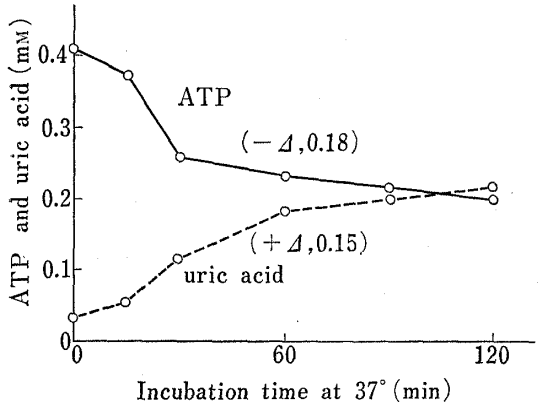

Fig. 3. Uric Acid Formation from ATP in Rat Blood

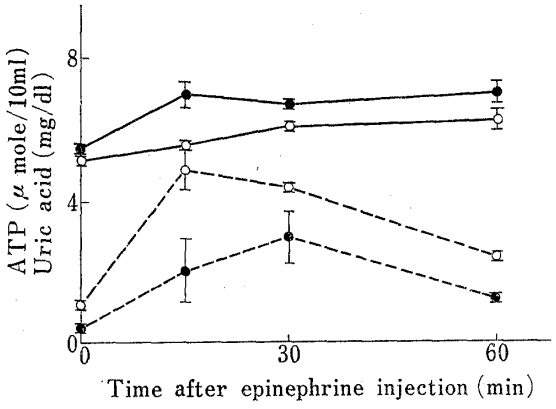

Fig. 4. Effect of *l*-Epinephrine on Blood ATP and Plasma Uric Acid in Rats

○: *l*-epinephrine, 1.0 mg/kg, administered intraperitoneally
●: allopurinol, 250 mg/kg, administered orally 24 hr before epinephrine
——: blood ATP, ------: plasma uric acid
Vertical lines represent standard error ($n=6$).

Relation between Blood ATP Level and Plasma Uric Acid

ATP in the blood was found the most part in blood cell fraction, while uric acid was contained in the plasma. In experiments *in vitro*, blood ATP was degraded by incubation at 37° giving an increase in the concentration of plasma uric acid. As shown in Fig. 3, the decrease in concentration of ATP well corresponded to the increase in uric acid, and this catabolism reached a steady state within half degradation of ATP, if the blood was incubated immediately after sacrifice of the rat. However, the concentration of blood ATP could be readily reduced to half without corresponding production of plasma uric acid, if the blood was placed in an ice-box. Blood which had been left in an ice-box for several hours showed recovery of ATP to its former level on incubation at 37°, in spite of there also being a marked increase in uric acid.

On the other hand, the blood from the rats which had received *l*-epinephrine showed a slight increase in ATP level which was emphasized in rats pretreated with allopurinol, as indicated in Fig. 4. From this evidence it seems likely that purine compounds in the blood are utilized either in catabolism to plasma uric acid or by incorporation into ATP in the blood cells.

Xanthine Oxodase Level in Mammalian Plasma

We could not deduce exactly whether the plasma uric acid arising in an extra-hepatic process was formed in the plasma or in the respective tissues, but the uric acid producing system in plasma is highly significant for the direct conversion of substrates from all tissues to plasma uric acid. Moreover, the species difference of this process should be investigated to evaluate whether it is significant in all species including man, as has been well known the difference of metabolic pathway for purine compounds in the liver.

As shown in Table IV, the activity of plasma xanthine oxidase shows a marked species difference, and is independent of the plasma level of uric acid in normal animals. However, this enzyme is undoubtedly present even in human plasma and the species difference corresponds to the amount of uric acid produced during incubation of the blood, as shown in Table V.

Table IV. Plasma Xanthine Oxidase Activity and Uric Acid Level in Mammals

Species	Relative activity	Plasma level of uric acid
Rat	100 (1.07…1.40)[a]	100 (0.86…1.87)[b]
Guinea pig	165	88
Mouse	43	184
Human	20	260
Dog	5	31

a) units/ml b) mg/dl

Table V. Increase of Plasma Uric Acid by Incubation of Blood

Object	Plasma uric acid (mg/dl)		
	Without incubation	With incubation	Increase
Rats (7w, male)	1.10	2.95	1.85
Mice (4w, male)	1.62	2.60	0.98
Human (male)			
A) (24y)	4.55	4.79	0.24
B) (34y)	5.52	5.88	0.36
C) (36y)	4.85	5.31	0.46
D) (49y)	7.36	7.69	0.33

Two ml of blood was incubated at 37° for 60 min.

A point of great interest is the relationship between the species difference of plasma xanthine oxidase level and the intensity of hyperuricemia elicited by *l*-epinephrine. This was studied in rodents, with which it is possible to establish constant experimental conditions. From the slope of the dose response curve taken at fifteen minutes after intraperitoneal injection of *l*-epinephrine, it was calculated that the degree of induction of hyperuricemic state in mice corresponds to about 35% of that in rats. This may possibly be attributable to the species difference in plasma xanthine oxidase level. However, the similar attribution was impossible for the effect of norepinephrine in mice and rats, and for the effect of epinephrine in rats and guinea pigs.

Discussion

Beginning in our previous communications and continuing in this report, we have presented evidence to support the proposition of a new mechanism for the induction of hyperuricemia, that is, the effect of excess of catecholamines, especially epinephrine leads to induction of a rapid reduction of ATP in various tissues and the subsequent catabolism of its metabolites to uric acid, not only in liver, but also in extra-hepatic system. Moreover, the present research has shown that in fact most of plasma uric acid elicited by epinephrine depends on an extra-hepatic process, including the reduction of intestinal ATP and the uric acid production in the plasma.

Although it is not possible to get direct evidence in this basic field, we expect that our consideration for the epinephrine-induced hyperuricemia is useful for the investigation of hyperuricemia in clinical field, where the stress-ful environment has been alarmed in the pathogenesis of hyperuricemia, and the α-acting catecholamines have shown the character of hyperuricemia induction which is considered to be due to the inhibitory effect in uric acid clearance.[9]

Our work so far still leaves certain aspects of the mechanism of epinephrine induction of hyperuricemia unexplained. First of all, we still lack detailed experimental evidence on the nature of the α-receptor to catecholamine. Various hypotheses have been made on catecholamine receptors,[10–12] but they do not provide explanation of all pharmacological aspects when catecholamines are administered in animals. We have based our present hypothesis on the receptor theory by Belleau, since this affords a favorable explanation of our experimental results. However, other explanations for the relation between the action of catecholamines and elevation of plasma uric acid are possible, for example, modification of intracellular reactions by hypoxia leading to degradation of ATP through the ATPase reaction. In any case, the reduction of tissue ATP levels is an important basis for the metabolic influence of catecholamines, and induction of hyperuricemia can be considered related to this reduction under more drastic conditions.

The next problem is to develop a way of prolonging the hyperuricemic state induced by epinephrine. Enhanced plasma uric acid levels elicited by *l*-epinephrine are not maintained for a long enough period to provide a model for pathological hyperuricemia in man. It may be that normal experimental animals, even when they are brought to a hyperuricemic state, can rapidly recover a normal state through the normal function in liver and kidney, including the further oxidation of uric acid to allantoin in liver and the clearance of uric acid in kidney. So for further progress along this line, it is necessary to prepare model animals, in which the liver or kidney function is disturbed. Our previous communication,[4] on the inhibitory effect of adrenochrome, a metabolite of epinephrine, suggests another reason why the effect is not pro-

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10) G.A. Robison, R.W. Butcher, and E.W. Sutherland, *Ann. N. Y. Acad. Sci.*, **139**, 703 (1967).
11) B. Belleau, *Pharmacol. Rev.*, **18**, 131 (1966).
12) J.R. Smythies, *Eur. J. Pharm.*, **19**, 18 (1972).

longed. If an auto-regulating process is present in the action mechanism of catecholamines in the receptor area, prolongation of hyperuricemic state will be not given in the experiments in normal animals.

Another problem requiring technical development is the determination of tissue ATP. We did not use the method of rapid freezing in order to avoid the influence of anesthetic, but we did ensure that tissues were homogenized within one minute of decapitation. As the results for ATP content were not very different from those obtained by rapid freezing, we believe that they do reflect the relative influence of epinephrine. However, we could not determine whether ATP was reduced by epinephrine in subcellular fractions. And although we cannot expect to obtain such information at present, it would lead to an elucidation of the relation between catecholamine receptors and reduction of ATP. The technical approach for the determination of tissue ATP will also give an information on the influence of epinephrine to blood vessels, in which a marked activity of ATPase has been reported.[13] Then, it may be reasonable to consider that, if we can determine the ATP level in blood vessels, the reduction of ATP in them by epinephrine treatment may also be recognized corresponding to the elevation of plasma uric acid.

13) G.S. Harris, *Eur. J. Pharm.*, **19**, 137 (1972).