Effect of Branched-Chain Amino Acids on the Plasma Concentration of Uridine Does Not Occur Via the Action of Glucagon or Insulin

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To examine whether branched-chain amino acids affect the plasma concentration of uridine, we administered branched-chain amino acids (L-isoleucine, 2.85 g, L-leucine 5.71 g, and L-valine, 3.43 g) orally to 6 healthy subjects. Plasma uridine and glucose decreased by 44% and 12%, respectively, together with an increase in plasma isoleucine, leucine, and valine 90 minutes after administration. However, branched-chain amino acids did not affect the plasma concentration and urinary excretion of purine bases (hypoxanthine, xanthine, and uric acid) and uridine or the plasma concentration of insulin, glucagon, and cyclic adenosine monophosphate (cAMP). Since small amounts of regular insulin, which were found to decrease plasma glucose more than the amino acids, did not decrease the plasma concentration of uridine, these results suggest that plasma uridine was decreased by a direct effect of the branched-chain amino acids on the cellular uptake and/or release of uridine.

RECENT STUDIES[1,2] have demonstrated that glucagon and dibutyryl cyclic adenosine monophosphate (cAMP) decrease the plasma concentration of uridine without an increase in the urinary excretion of uridine. In a previous study,[3] glucagon and dibutyryl cAMP enhanced uridine uptake into cells via the Na-dependent nucleoside transport pathway in vitro, suggesting that glucagon stimulates the production of cAMP, thereby enhancing the uptake of uridine into cells. In addition, intravenous administration of a 12% amino acid solution increased plasma glucagon and decreased plasma uridine, although it did not increase plasma cAMP.[4] These results suggest that glucagon secreted from α cells by amino acids has an important role in decreasing the plasma concentration of uridine, although the secreted glucagon is not sufficient to increase the plasma concentration of cAMP in the peripheral blood. The physiological role of the Na-dependent nucleoside transport pathway is to preserve extracellular nucleoside for the endogenous synthesis of nucleic acids,[3,5] since this pathway is enhanced in response to a mitogenic stimulus as in partial hepatectomy.[5] Therefore, the enhancement of nucleoside transport by the amino acid–induced secretion of glucagon may have a clinically profitable role in nucleic acid biosynthesis in the physiological situation leading to hypertrophia and hyperplasia of the liver. However, the possibility that amino acids may directly decrease the plasma concentration of uridine cannot be excluded.

Previous studies[6-8] have demonstrated that branched-chain amino acids are effective in the treatment of hepatic coma in patients with hepatic encephalopathy, the improvement of protein metabolism in acute hepatic failure in rats, and the protection of the heart from myocardial ischemic injury in rats. Another study[9] suggested that branched-chain amino acids may be important in potentiating the effect of insulin on the inhibition of protein breakdown and promoting protein synthesis. However, it remains undetermined as to whether branched-chain amino acids affect nucleoside metabolism, including uridine, although an investigation of this question would be of clinical importance. Therefore, we examined whether branched-chain amino acids affect the plasma concentration of uridine by using small amounts of branched-chain amino acids that do not significantly stimulate the secretion of glucagon.

MATERIALS AND METHODS

Subjects and Protocol

After provision of informed consent, we performed the first study on 6 healthy subjects (body weight, 50 to 75 kg) aged 32 to 50 years. They had normal laboratory data including a complete blood cell count, serum aspartate transaminase, alanine transaminase, and creatinine, and fasting plasma glucose and hemoglobin A_{1c} (data not shown). After an overnight fast except for water, 1-hour urine was collected 3 times over 3 hours and blood was drawn at the midpoint of each urinary collection by heparinized syringes. The subjects ingested amino acids (L-isoleucine 2.85 g, L-leucine, 5.71 g; and L-valine 3.43 g) together with 200 mL water after the first 1-hour urine collection. Two weeks later, the second study was performed. After an overnight fast, 1-hour urine was collected 3 times over 3 hours and blood was drawn at the midpoint of each urinary collection by heparinized syringes. After the first urine collection, regular insulin (0.04 to 0.07 U/kg weight) was administered subcutaneously.

Blood and Urine Analyses

Plasma concentrations of uridine, hypoxanthine, and xanthine were determined by high-performance liquid chromatography (HPLC) as described previously.[10] Urinary concentrations of hypoxanthine and xanthine were also determined by HPLC as described previously.[10] The urinary concentration of uridine was determined by HPLC with column-switching as described previously.[4] In brief, the chromatograph consisted of 2 CCPM pumps (Tosoh, Tokyo, Japan), a SC-8020 system controller (Tosoh), 2 spectrophotometric detectors (UV-8010 and UV-8020; Tosoh), and VC-8020 column-switching valves (Tosoh). The chromatographic columns were a Wakosil 5C18-200 (4.6 × 250 mm; Wako Pure Chemicals, Osaka, Japan) as the first column and a Tosoh TSK Gel (ODS-120A; 4.6 × 250 mm) as the second column. In both columns, the mobile phase was 20 mmol/L KH_2PO_4 (pH 2.2), the flow rate 1 mL/min, and the detection wavelength 254 nm. Twenty microliters of urine without dilution were applied to the first column. At the fraction time in which the uridine was eluted via the first column, the 2 columns were connected and the eluate from the second column was monitored.

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Submitted March 11, 1999; accepted June 23, 1999.
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0026-0495/00/4901-0002$10.00/0

Table 1. Effect of Amino Acids on the Plasma Concentration of Purine Bases and Uridine (N = 6)

Parameter	−30 min	30 min	90 min
Hypoxanthine	0.94 ± 0.34	1.20 ± 0.42	1.02 ± 0.60
Xanthine	0.72 ± 0.22	0.96 ± 0.50	0.72 ± 0.38
Uric acid	370 ± 60	370 ± 61	368 ± 61
Uridine	4.93 ± 0.66	4.58 ± 0.88	2.76 ± 0.70*

NOTE. Values are the mean ± SD (μmol/L). −30, 30, and 90 min denote 30 minutes before and 30 and 90 minutes after administration of branched-chain amino acids, respectively.
*$P < .01$.

Plasma and urinary concentrations of uric acid were measured by the uricase method using an autoanalyzer. Lactic acid and pyruvic acid in blood and insulin, glucagon, cAMP, and inorganic phosphate in plasma were determined as described previously.[1,2,4] Plasma concentrations of amino acids were also determined as described previously.[11]

Chemicals

Uridine, hypoxanthine, xanthine, and uric acid were purchased from Sigma Chemical (St Louis, MO). Other chemicals were obtained from Wako Pure Chemicals.

Statistical Analysis

Values are the mean ± SD. The significance of differences between means was analyzed by the two-tailed *t* test.

RESULTS

Effect of Amino Acid Intake on Plasma Concentration of Purine Bases and Uridine

Amino acids did not affect the plasma concentration of hypoxanthine, xanthine, or uric acid during the study. In contrast, plasma uridine was decreased by 44% at 90 minutes after intake of the amino acids as compared with the control value at 30 minutes before intake (Table 1 and Fig 1).

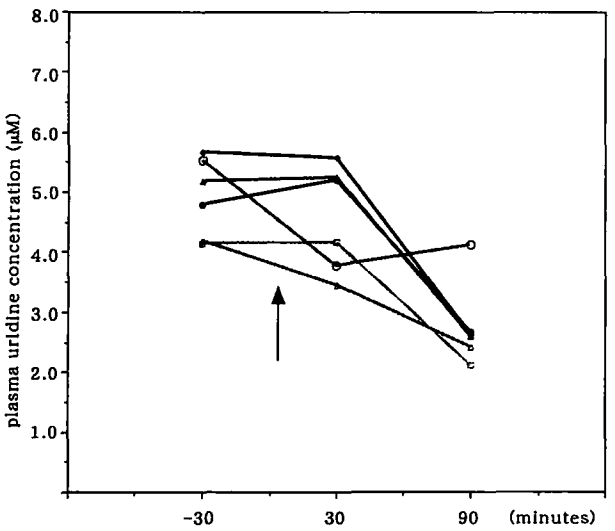

Fig 1. Effect of branched-chain amino acids on the plasma concentration of uridine. Arrow indicates amino acid intake.

Table 2. Effect of Amino Acids on the Urinary Excretion of Purine Bases and Uridine (N = 6)

Parameter	1	2	3
Hypoxanthine	8.44 ± 2.46	8.52 ± 3.42	7.01 ± 2.91
Xanthine	6.60 ± 2.51	6.68 ± 2.81	6.13 ± 3.84
Uric acid	330 ± 110	310 ± 111	299 ± 107
Uridine	0.26 ± 0.06	0.23 ± 0.05	0.22 ± 0.08

NOTE. Values are the mean ± SD (μmol/mmol creatinine). 1, 2, and 3 denote the 1-hour period before administration of branched-chain amino acids, the 1-hour period after administration of branched-chain amino acids, and the 1-hour period following period 2, respectively.

Effect of Amino Acid Intake on Urinary Excretion and Fractional Clearance of Purine Bases and Uridine and Clearance of Creatinine

Amino acids did not affect the urinary excretion of hypoxanthine, xanthine, uric acid, or uridine during the study (Table 2). Neither the clearance of creatinine nor the fractional clearance of hypoxanthine, xanthine, uric acid, and uridine were affected by the intake of amino acids (Table 3).

Effect of Amino Acid Intake on Glucagon, Insulin, cAMP, Glucose, and Inorganic Phosphate in Plasma and Lactic Acid and Pyruvic Acid in Blood

Plasma concentrations of insulin, glucagon, and cAMP were not affected by the intake of amino acids. In contrast, plasma glucose was decreased by 12% at 90 minutes after the intake of amino acids as compared with the control value at 30 minutes before intake. The plasma concentration of inorganic phosphate was also decreased by 16% at 90 minutes after intake. Amino acids did not affect the blood concentration of lactic acid, but decreased blood pyruvic acid by 41% at 90 minutes after intake (Table 4).

Effect of Amino Acid Intake on Plasma Leucine, Isoleucine, and Valine

Plasma concentrations of leucine, isoleucine, and valine increased by 3.9-fold, 3.7-fold, and 2.5-fold, respectively, at 30 minutes and by 6.6-fold, 6.5-fold, and 4.3-fold at 90 minutes

Table 3. Effect of Amino Acids on the Clearance of Creatinine and Fractional Clearance of Purine Bases and Uridine (N = 6)

Parameter	1	2	3
Creatinine clearance (mL/min)	103.7 ± 8.6	107.1 ± 12.0	101.6 ± 4.8
Fractional clearance (mL/min/mL/min × 100)			
Hypoxanthine	74.5 ± 26.9	60.0 ± 24.1	63.4 ± 31.3
Xanthine	70.2 ± 26.1	63.3 ± 32.9	70.6 ± 40.6
Uric acid	6.87 ± 2.03	6.76 ± 2.65	6.27 ± 2.51
Uridine	0.41 ± 0.09	0.41 ± 0.15	0.64 ± 0.29

NOTE. Values are the mean ± SD. 1, 2, and 3 denote the 1-hour period before administration of branched-chain amino acids, the 1-hour period after administration of branched-chain amino acids, and the 1-hour period following period 2, respectively. Fractional clearance of the respective parameters denotes the parameter clearance/creatinine clearance × 100.

Table 4. Effect of Amino Acids on the Concentration of Glucagon, Insulin, cAMP, and Inorganic Phosphate in Plasma and Glucose, Lactic Acid, and Pyruvic Acid in Blood (N = 6)

Parameter	−30 min	30 min	90 min
Glucagon	107 ± 52	125 ± 51	123 ± 45
Insulin	6.7 ± 2.2	—	7.4 ± 2.3
Cyclic AMP	12.3 ± 2.1	—	12.8 ± 2.6
Inorganic phosphate	0.97 ± 0.13	0.94 ± 0.16	0.81 ± 0.13†
Glucose	5.44 ± 0.40	5.42 ± 0.44	4.81 ± 0.34*
Lactic acid	0.91 ± 0.39	0.79 ± 0.33	0.69 ± 0.44
Pyruvic acid	0.069 ± 0.033	0.056 ± 0.013	0.041 ± 0.010†

NOTE. Values are the mean ± SD in μmol/L, except for insulin (μU/mL), glucagon (pg/mL), and cAMP (nmol/L). −30, 30, and 90 min denote 30 minutes before and 30 and 90 minutes after administration of branched-chain amino acids, respectively.

*$P < .05$.

†$P < .01$.

after intake as compared with the respective control values (Table 5).

Effect of Insulin Injection on the Plasma Concentration, Urinary Excretion, and Fractional Clearance of Purine Bases and Uridine and the Clearance of Creatinine

Subcutaneous administration of insulin did not affect the plasma concentration, urinary excretion, or fractional clearance of purine bases and uridine or the clearance of creatinine (Tables 6, 7, and 8).

Effect of Insulin Injection on Glucagon, Insulin, Glucose, and Inorganic Phosphate in Plasma and Lactic Acid and Pyruvic Acid in Blood

The plasma concentration of insulin increased by 5.4-, 5.1-, and 3.3-fold at 30, 60, and 90 minutes, respectively, after administration of insulin as compared with the control value at 30 minutes before administration of insulin. However, the concentrations of glucagon, glucose, and inorganic acid in plasma and lactic acid and pyruvic acid in blood were not affected (Table 9).

DISCUSSION

In the present study, oral administration of branched-chain amino acids (L-isoleucine 2.85 g, L-leucine, 5.71 g, and L-valine 3.43 g) increased the plasma concentration of isoleucine, leucine, and valine (Table 5) and decreased the plasma concentration of uridine (Fig 1 and Table 1) but did not significantly affect the urinary excretion or fractional clearance of uridine

Table 5. Effect of Branched-Chain Amino Acids (L-isoleucine 2.85 g, L-leucine 5.71 g, and L-valine 3.43 g) on the Plasma Concentration of Valine, Isoleucine, and Leucine (N = 6)

Parameter	−30 min	30 min	90 min
Leucine	158 ± 15	610 ± 115*	1,048 ± 79*
Isoleucine	82 ± 6	302 ± 52*	533 ± 31*
Valine	260 ± 26	643 ± 88*	1,118 ± 60*

NOTE. Values are the mean ± SD (μmol/L). −30, 30, and 90 min denote 30 minutes before and 30 and 90 minutes after administration of branched-chain amino acids, respectively.

*$P < .01$.

Table 6. Effect of Insulin on the Plasma Concentration of Purine Bases and Uridine (N = 6)

Parameter	−30 min	30 min	60 min	90 min
Hypoxanthine	1.06 ± 0.45	1.10 ± 0.45	1.05 ± 0.42	1.08 ± 0.43
Xanthine	0.82 ± 0.22	0.76 ± 0.18	0.72 ± 0.20	0.72 ± 0.22
Uric acid	339 ± 32	333 ± 36	333 ± 35	333 ± 37
Uridine	4.70 ± 0.66	4.50 ± 0.56	4.42 ± 0.62	4.44 ± 0.72

NOTE. Values are the mean ± SD (μmol/L). −30, 30, 60, and 90 min denote 30 minutes before and 30, 60, and 90 minutes after administration of insulin, respectively.

(Table 3). Previous studies[12-15] have demonstrated that enhanced adenosine triphosphate degradation increases the degradation of uracil nucleotides, leading to an increased plasma concentration of uridine. Therefore, a decrease in the plasma concentration of uridine may be related to purine metabolism. However, the present study indicates that branched-chain amino acids did not affect purine metabolism, because there were no changes in the plasma concentration or urinary excretion of purine bases (hypoxanthine, xanthine, and uric acid) (Tables 1 and 2). Accordingly, other factor(s) must be considered as the cause of the amino acid–induced decrease in the plasma concentration of uridine.

One such factor is glucagon. Since amino acids enhance the secretion of glucagon, the increased plasma concentration of glucagon in the portal vein may accelerate the uptake of uridine via the Na-dependent nucleoside transport pathway in the liver and increase glucose and pyruvic acid as described previously.[4] However, the present study demonstrates that branched-chain amino acids did not increase the plasma concentration of glucagon, cAMP, glucose, or pyruvic acid in peripheral blood (Table 4), suggesting that they decrease the plasma concentration of uridine independently of the action of glucagon. Another factor is insulin. The secretion of insulin is stimulated by amino acids, and insulin may also decrease the plasma concentration of uridine.[3,16] The increased plasma concentration of insulin in the portal vein may accelerate the uptake of uridine via the Na-dependent nucleoside pathway in the liver, since insulin enhances the uptake of uridine into liver cells via this pathway along with the synthesis of ribonucleotides.[3,16] In the present study, the concentration of glucose was significantly decreased by amino acid ingestion despite an unchanged plasma insulin level (Table 4). A decrease in the plasma concentration of glucose seems ascribable to an amino acid–stimulated secretion of insulin from the pancreas. Of course, the increase in the concentration of insulin in the portal vein is not sufficient to increase the plasma concentration of insulin in the peripheral

Table 7. Effect of Insulin on the Urinary Excretion of Purine Bases and Uridine (N = 6)

Parameter	1	2	3
Hypoxanthine	7.18 ± 1.24	7.29 ± 0.31	6.92 ± 0.064
Xanthine	5.42 ± 1.42	5.06 ± 1.23	5.38 ± 1.57
Uric acid	264 ± 71	264 ± 50	240 ± 31
Uridine	0.23 ± 0.06	0.023 ± 0.06	0.21 ± 0.05

NOTE. Values are the mean ± SD (μmol/mmol creatinine). 1, 2, and 3 denote the 1-hour period before administration of insulin, the 1-hour period after administration of insulin, and the 1-hour period following period 2, respectively.

Table 8. Effect of Insulin on the Clearance of Creatinine and Fractional Clearance of Purine Bases and Uridine (N = 6)

Parameter	1	2	3
Creatinine clearance (mL/min)	107.2 ± 6.7	109.2 ± 6.3	108.0 ± 8.6
Fractional clearance			
Hypoxanthine	75.0 ± 43.4	70.2 ± 38.8	70.8 ± 45.8
Xanthine	63.2 ± 30.2	70.1 ± 27.9	76.1 ± 51.0
Uric acid	7.03 ± 2.44	7.06 ± 1.33	6.44 ± 1.21
Uridine	0.42 ± 0.11	0.45 ± 0.11	0.42 ± 0.11

NOTE. Values are the mean ± SD. Fractional clearance of the respective parameters denotes the parameter clearance/creatinine clearance × 100.

Table 9. Effect of Insulin on the Plasma Concentration of Insulin, Glucagon, and Glucose (N = 6)

Parameter	−30 min	30 min	60 min	90 min
Insulin	5.2 ± 2.0	28.0 ± 8.5*	26.3 ± 5.3*	16.9 ± 6.1*
Glucagon	114 ± 19	109 ± 12	123 ± 11	128 ± 12
Glucose	5.64 ± 0.10	4.57 ± 0.60*	4.36 ± 0.83*	4.52 ± 0.59*
Lactic acid	0.80 ± 0.34	0.76 ± 0.29	—	0.79 ± 0.27
Pyruvic acid	0.051 ± 0.011	0.047 ± 0.013	—	0.048 ± 0.015
Inorganic phosphate	0.90 ± 0.18	0.90 ± 0.15	0.90 ± 0.15	0.90 ± 0.15

NOTE. Values are the mean ± SD in μmol/L, except for insulin (μU/mL) and glucagon (pg/mL). −30, 30, 60, and 90 min denote 30 minutes before and 30, 60, and 90 minutes after administration of insulin, respectively.

*$P < .01$.

blood, since much of the insulin secreted from β cells into the portal vein is taken up by the liver.

To investigate whether a decrease in the plasma concentration of uridine relates to a subtle decrease in the plasma concentration of glucose, we administered subcutaneous small amounts of insulin that decreased the plasma concentration of glucose more than the branched-chain amino acids. Insulin did not decrease uridine or inorganic acid in plasma or pyruvic acid in blood (Tables 6 and 9), suggesting that the branched-chain amino acid–induced decrease in uridine and inorganic phosphate in plasma and pyruvic acid in blood is not ascribable to insulin or insulin-induced hypoglycemia. Therefore, the branched-chain amino acid–induced decrease in the plasma concentration of uridine may result from an effect of the branched-chain amino acids directly or via an unknown factor. In either case, since the urinary excretion and fractional clearance of uridine were not significantly affected by branched-chain amino acids, it is suggested that branched-chain amino acids enhance the cellular uptake of uridine and/or decrease the cellular output of uridine, resulting in a decrease in its plasma concentration.

Branched-chain amino acids are used to reverse hepatic coma[6,17] and to improve the nutritional status and respiratory muscle function in chronic obstructive pulmonary disease.[18] They are also suggested to be important in potentiating the effect of insulin by inhibiting protein breakdown and promoting protein synthesis in acute renal failure.[9] In addition to these actions, the present study suggests that branched-chain amino acids have a role in nucleoside metabolism. However, it is undetermined as to whether they play a significant role in intracellular nucleoside metabolism as do glucagon and insulin. Further, it is undetermined as to how branched-chain amino acids decrease the plasma concentration of uridine. In addition, although an amino acid–induced 50% increase in the fractional clearance of uridine was not statistically demonstrated (Table 3), it cannot be ruled out because of the small sample size (6 subjects). Therefore, further investigation is needed.

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