Effect of Ethanol and Fructose on Plasma Uridine and Purine Bases

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To determine whether both ethanol and fructose increase the plasma concentration of uridine, we administered ethanol (0.6 g/kg) or fructose (1.0 g/kg) to seven normal subjects. Both ethanol and fructose increased the plasma concentration of uridine together with an increase in the plasma concentration of oxypurines, whereas fructose also increased the plasma concentration of uric acid, but ethanol did not. In ethanol ingestion and fructose infusion, an increase in the plasma concentration of purine bases correlated with that of uridine. These results strongly suggest that an increase in the plasma concentration of uridine is ascribable to increased pyrimidine degradation following purine degradation increased by ethanol and fructose.

IN PREVIOUS STUDIES,[1-3] it has been shown that ischemia increases both pyrimidine and purine degradation because aerobic glycolysis is inhibited. As a result, uridine (a degradation product of pyrimidine nucleotides) is leaked into blood together with hypoxanthine (a degradation product of purine nucleotides). Increases in the blood concentration and urinary excretion of these degradation products express the degree of ischemia-induced purine degradation and pyrimidine degradation.[4] Namely ischemia disturbs the production of adenosine triphosphate (ATP) in the tricarboxylic acid cycle, leading to an increase in the concentration of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) in cells and a decrease in ATP. Increases in the concentration of AMP and ADP with a decrease in ATP accelerate the conversion of AMP to inosine monophosphate (IMP), followed by enhanced purine degradation. Furthermore, since uridine diphosphate (UDP) is phosphorylated to uridine triphosphate (UTP) using ATP, a decrease in the concentration of ATP leads to an increase in the concentration of UDP and uridine monophosphate (UMP), resulting in the accelerated conversion of UMP to uridine. These results suggest that abrupt ATP consumption induced by any substance induces pyrimidine degradation. Therefore, in the present study, we investigated whether the plasma concentration of uridine is increased by ethanol[4] and fructose,[5] which consume ATP. In addition, to investigate whether an increase in the plasma concentration of uridine reflects purine degradation induced by ethanol or fructose (Fig 1), we administered to normal subjects both ethanol and fructose and compared fructose-induced increases in the plasma concentration and urinary excretion of uridine and purine bases with those induced by ethanol.

SUBJECTS AND METHODS

Chemicals

Hypoxanthine, xanthine, and uridine were purchased from Wako Pure Chemical Industries (Osaka, Japan), and 10% fructose solution was obtained from Otsuka Pharmaceuticals (Tokyo, Japan). Other chemicals were obtained from Wako Pure Chemical Industries.

Subjects and Protocol

The first study was conducted on seven healthy men aged 30 to 48 years and weighing 51 to 60 kg. The subjects had normal laboratory data. After informed consent was obtained, they ingested 300 mL distilled water containing ethanol 0.6 g/kg body weight over 2 minutes after an overnight fast. Urine was completely voided 1 hour before ethanol ingestion, and then urine was collected at an interval of 1 hour two times. Blood samples were drawn with heparinized syringes 30 minutes before ethanol ingestion and then again 30 minutes and 1 hour after the beginning of ethanol ingestion. During the study, water was not restricted. The second study was also conducted on the same subjects 2 weeks later. Fructose 1 g/kg body weight was infused over 1 hour as a 10% solution after an overnight fast. Urine was completely voided 1 hour before the beginning of fructose infusion, and then urine was collected at an interval of 1 hour two times. Blood samples were drawn with heparinized syringes 30 minutes before the beginning of fructose infusion and then again 30 minutes and 1 hour after beginning fructose infusion.

Blood and Urine Analyses

Plasma and urinary concentrations of hypoxanthine, xanthine, and uridine were determined by the method used by Yamamoto et al[6] using high-performance liquid chromatography as described previously. The column was a Wakosil 5C-18 (4.6 mm inner diameter × 250 mm; Wako Pure Chemical Industries). The uric acid level in plasma and urine was measured by the uricase method using an autoanalyzer. Blood concentrations of lactic acid and pyruvic acid were measured by enzymatic methods using a Determinar LA kit (Kiyowa Medix, Tokyo, Japan) and a Determinar PA kit (Kiyowa Medix), respectively. Plasma concentrations of ethanol and fructose were also determined by enzymatic methods using an F-kit ethanol (Boehringer, Mannheim, Germany) and an F-kit sucrose/glucose/fructose (Boehringer), respectively.

Statistics

Values are expressed as the mean ± SE. The significance of differences was assessed by ANOVA for all variables. $P < .05$ was considered statistically significant.

RESULTS

Plasma Concentrations of Ethanol on Ethanol Ingestion and of Fructose on Fructose Infusion

Plasma concentrations of ethanol were less than the detection limit, 744 ± 21 μg/mL, and 796 ± 45 μg/mL 30 minutes before, 30 minutes after, and 1 hour after beginning ethanol ingestion, respectively. Plasma concentrations of fructose were also less

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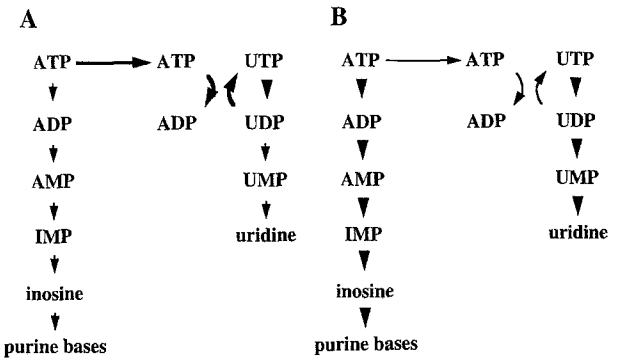

Fig 1. Scheme of pyrimidine and purine degradation induced by ethanol and fructose. (A) Before administration of ethanol or fructose; (B) after or during administration of ethanol or fructose. Thick downward arrows indicate increased degradation.

than the detection limit, 3.7 ± 0.3 mmol/L, and 3.6 ± 0.2 mmol/L 30 minutes before, 30 minutes after, and 1 hour after beginning fructose infusion, respectively.

Effect of Ethanol or Fructose on Plasma Concentrations of Uridine, Hypoxanthine, Xanthine, and Uric Acid

Ethanol increased the plasma concentration of hypoxanthine 3.3-fold, xanthine 4.1-fold, and uridine 1.2-fold 30 minutes after the beginning of ethanol ingestion, and it also increased plasma concentrations of hypoxanthine, xanthine, and uridine 2.2-fold, 5.2-fold, and 1.3-fold, respectively, 1 hour after beginning ethanol ingestion, as compared with the respective values 30 minutes before ethanol ingestion (Table 1). However, ethanol did not affect the plasma concentration of uric acid 30 minutes or 1 hour after beginning ethanol ingestion (Table 1). Fructose increased the plasma concentration of xanthine 2.2-fold, uric acid 1.1-fold, and uridine 1.7-fold 30 minutes after the beginning of fructose infusion (Table 1), and it also increased plasma concentrations of hypoxanthine, xanthine, uric acid, and uridine 2.0-fold, 2.1-fold, 1.1-fold, and 1.7-fold, respectively, 1 hour after beginning fructose infusion, as compared with the respective values 30 minutes before fructose infusion (Table 1). Plasma concentrations of xanthine 30 minutes and 1 hour after ethanol ingestion were higher than those 30 minutes and 1 hour after fructose infusion, respectively, although the plasma concentration of xanthine was not different for before ethanol ingestion versus before fructose infusion (Table 1). In contrast, plasma concentrations of uridine 30 minutes and 1 hour after beginning fructose were higher than those 30 minutes and 1 hour after beginning ethanol ingestion, although the plasma concentration of uridine was not different for before fructose infusion versus before ethanol ingestion (Table 1). However, plasma concentrations of hypoxanthine and uric acid were not different between ethanol ingestion and fructose infusion during the study.

Table 1. Plasma Concentrations of Hypoxanthine, Xanthine, Uric Acid, and Uridine (μmol/L, mean ± SE)

Parameter	Period 1	Period 2	Period 3
Ethanol ingestion			
Hypoxanthine	0.87 ± 0.08	2.84 ± 0.74*	1.88 ± 0.41*
Xanthine	0.62 ± 0.03	2.56 ± 0.47†‡	3.22 ± 0.83*‡
Uric acid	342 ± 10	346 ± 11	349 ± 13
Uridine	3.84 ± 0.14	4.60 ± 0.13†§	4.88 ± 0.27*§
Fructose infusion			
Hypoxanthine	0.96 ± 0.08	2.62 ± 0.91	1.89 ± 0.22*
Xanthine	0.53 ± 0.04	1.18 ± 0.13†	1.10 ± 0.06†
Uric acid	349 ± 11	368 ± 13†	374 ± 13†
Uridine	4.13 ± 0.13	7.02 ± 0.74*	6.88 ± 0.43†

NOTE. Period 1, 30 minutes before beginning ethanol ingestion or fructose infusion; 2, 30 minutes after beginning ethanol ingestion or fructose infusion; 3, 1 hour after beginning ethanol ingestion or fructose infusion.

*$P < .05$, †$P < .01$: *v* period 1.

‡$P < .05$, and §$P < .01$: *v* fructose infusion.

Effect of Ethanol or Fructose on Urinary Excretion of Hypoxanthine, Xanthine, and Uric Acid

Ethanol increased the 1-hour urinary excretion of hypoxanthine and xanthine 3.7-fold and 4.5-fold, respectively, as compared with the respective values before ethanol ingestion (Table 2). However, ethanol did not affect the 1-hour urinary excretion of uric acid (Table 2). Fructose also increased the 1-hour urinary excretion of hypoxanthine and xanthine 3.6-fold and 1.9-fold, respectively, compared with the respective values before fructose infusion (Table 2), but did not affect urinary excretion of uric acid. Urinary excretion of uridine was negligible 1 hour after and 1 hour before ethanol ingestion, whereas it was 12.7 ± 2.8 μmol/h 1 hour after beginning fructose, although it was negligible 1 hour before fructose infusion (Table 2). The 1-hour urinary excretion of xanthine after beginning ethanol ingestion was more than after beginning fructose infusion, although 1-hour urinary excretion of xanthine was not different for before ethanol ingestion versus before fructose infusion (Table 2). The 1-hour urinary excretion of hypoxanthine and uric acid was not different for after ethanol ingestion versus after fructose infusion or for before ethanol ingestion versus before fructose infusion (Table 2).

Table 2. Urinary Excretion of Purine Bases and Uridine (μmol/h, mean ± SE)

Parameter	Period 1	Period 2
Ethanol ingestion		
Hypoxanthine	5.52 ± 0.36	20.38 ± 5.66*
Xanthine	3.71 ± 0.33	16.76 ± 3.23†
Uric acid	141 ± 6	157 ± 12
Uridine	ND	ND
Fructose infusion		
Hypoxanthine	5.28 ± 0.3	19.07 ± 3.28†
Xanthine	4.23 ± 0.36	7.86 ± 0.82*
Uric acid	144 ± 7	146 ± 11
Uridine	ND	11.7 ± 2.6†

NOTE. Period 1, 1 hour before beginning ethanol ingestion or fructose infusion; 2, 1 hour after beginning ethanol ingestion or fructose infusion.

Abbreviation: ND, not detected.

*$P < .05$ *v* period 1.

†$P < .01$ *v* period 1.

‡$P < .05$ *v* fructose infusion.

Relationship Between an Increase in the Plasma Concentration of Purine Bases (hypoxanthine + xanthine + uric acid) and That of Uridine

The ethanol-induced increase in the plasma concentration of purine bases correlated well with that of uridine using respective values 30 minutes and 1 hour after the beginning of ethanol ingestion ($r = .84$, $P < .01$). The fructose-induced increase in the plasma concentration of purine bases also correlated with that of uridine using respective values 30 minutes and 1 hour after the beginning of fructose infusion ($r = .69$, $P < .05$). Furthermore, with ethanol ingestion and fructose infusion, an increase in the plasma concentration of purine bases correlated with that of uridine ($r = .83$, $P < .01$; Fig 2).

Blood Concentrations of Inorganic Phosphate, Lactic Acid, and Pyruvic Acid

Both ethanol and fructose decreased the plasma concentration of inorganic phosphate 0.94-fold and 0.86-fold, respectively, 30 minutes after the beginning of ethanol ingestion or the beginning of fructose infusion, and 0.93-fold and 0.86-fold, respectively, 1 hour after the beginning of ethanol ingestion or fructose infusion (Table 3). Ethanol increased the blood concentration of lactic acid 1.4-fold 30 minutes after beginning ethanol ingestion, but this effect did not increase further at 1 hour after beginning ethanol ingestion, whereas fructose increased it 3.0-fold 30 minutes after and 2.9-fold 1 hour after beginning fructose infusion, as compared with the respective values before beginning ethanol ingestion or fructose infusion (Table 3). Ethanol decreased the blood concentration of pyruvic acid 0.4-fold 30 minutes after and 0.4-fold 1 hour after the beginning of ethanol ingestion, whereas fructose increased it 2.7-fold 30 minutes after and 2.8-fold 1 hour after the beginning of fructose infusion, as compared with the respective values before ethanol ingestion or fructose infusion (Table 3).

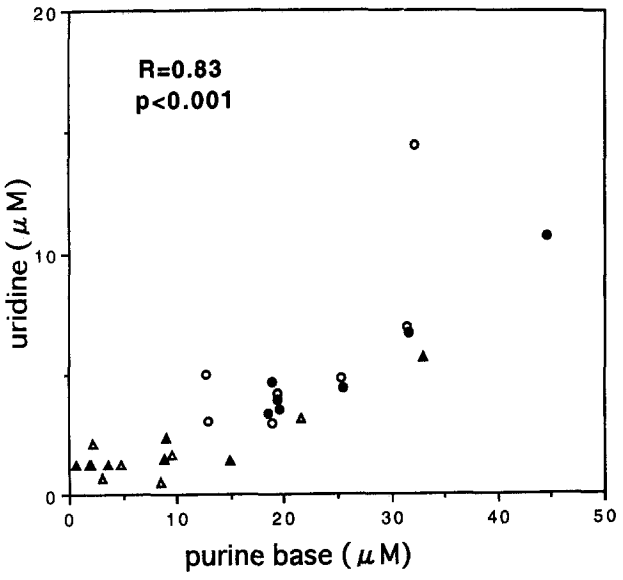

Fig 2. Relationship between plasma uridine increase and purine base increase. (○) Increased values 30 minutes after beginning fructose infusion; (●) increased values 1 hour after beginning fructose infusion; (△) increased values 30 minutes after beginning ethanol ingestion; (▲) increased values 1 hour after beginning ethanol ingestion.

Table 3. Blood Concentrations of Lactic Acid and Pyruvic Acid

Parameter	Period 1	Period 2	Period 3
Ethanol ingestion			
Inorganic phosphate	1.11 ± 0.03	1.04 ± 0.03*	1.03 ± 0.03*
Lactic acid	0.88 ± 0.12	1.23 ± 0.06*	1.18 ± 0.03
Pyruvic acid	69 ± 13	31 ± 4*	31 ± 7*
Fructose infusion			
Inorganic phosphate	1.11 ± 0.03	0.96 ± 0.03†	0.95 ± 0.04†
Lactic acid	0.77 ± 0.09	2.34 ± 0.25†	2.20 ± 0.18†
Pyruvic acid	60 ± 5	164 ± 21†	165 ± 22†

NOTE. Periods and statistical symbols are the same as in Table 1. Values are the mean ± SE (mmol/L for lactic acid, μmol/L for pyruvic acid, and mmol/L for inorganic phosphate).

DISCUSSION

In the present study, both ethanol and fructose increased the plasma concentration of uridine together with an increase in the plasma concentration of oxypurines, whereas fructose also increased the plasma concentration of uric acid, but ethanol did not (Table 1). In addition, increases in the plasma concentration and urinary excretion of xanthine induced by ethanol were more than those induced by fructose (Tables 1 and 2), but an increase in the plasma concentration of uridine induced by ethanol was less than that induced by fructose (Table 1). However, in ethanol ingestion and fructose infusion, an increase in the plasma concentration of purine bases correlated with that of uridine (Fig 2). Many previous studies[7-11] have demonstrated that ethanol accelerates purine degradation. On the other hand, a previous study[5] demonstrated that ethanol inhibited the activity of xanthine dehydrogenase, presumably by the following mechanism. Ethanol is oxidized to acetic acid via acetaldehyde, being coupled with the conversion of NAD to NADH. Since NADH is an inhibitor of xanthine dehydrogenase,[12,13] an ethanol-induced increase in the cytosolic concentration of NADH inhibits xanthine dehydrogenase activity. This action may play a role in ethanol-induced increases in the plasma concentration and urinary excretion of oxypurines. Therefore, we compared ethanol-induced increases in the plasma concentration and urinary excretion of purine bases (hypoxanthine, xanthine, and uric acid) with those induced by fructose to investigate whether ethanol-induced inhibition of xanthine dehydrogenase participates in the production of oxypurines to any degree. In addition, we compared ethanol- and fructose-induced increases in the plasma concentration of uridine with those of purine bases induced by ethanol and fructose to investigate whether an increase in the plasma concentration (a marker of pyrimidine degradation) correlates with that of purine bases (a marker of ethanol-induced purine degradation). Ethanol- or fructose-induced purine degradation has been suggested to be due to a loss of adenine nucleotides due to excessive organic phosphorylation using ATP as a phosphate donor.[4,5] In the present study, an ethanol- or fructose-induced decrease in the plasma concentration of inorganic phosphate (Table 3) also

suggested this mechanism. An ethanol- or fructose-induced decrease in ATP may accelerate degradation of pyrimidine nucleotides and thereby increase uridine production, because pyrimidine nucleotides are synthesized partly using ATP.[2] In the present study, a fructose-induced increase in the blood concentration of lactic acid may partly play a role in an increase in the plasma concentration of uric acid, since lactic acid inhibits urinary excretion of uric acid but does not inhibit that of oxypurines.[14] However, fructose increased the plasma concentration and urinary excretion of uridine and the plasma concentration and urinary excretion of oxypurines (Tables 1 and 2), indicating that fructose accelerated pyrimidine degradation and purine degradation. Therefore, an increase in the plasma concentration of uridine by fructose and in the plasma concentration of purine bases seems to reflect a degree of purine degradation by fructose (Fig 2). In contrast, ethanol increased plasma concentrations of uridine and oxypurines and urinary excretion of oxypurines (Tables 1 and 2), but did not increase the plasma concentration of uric acid (Table 1). Furthermore, an increase in the plasma concentration of uridine by ethanol was less than that induced by fructose; nevertheless, an increase in the plasma concentration of xanthine by ethanol was more than that induced by fructose (Table 1). These results suggested that ethanol inhibited the activity of xanthine dehydrogenase by an increase in NADH in the cytosol, because ethanol increases the ratio of lactic acid to pyruvic acid in blood, reflecting the ratio of NAD/NADH in the cytosol (Table 3), and it was also suggested that ethanol-induced inhibition of xanthine dehydrogenase plays a role in an increase in the plasma concentration and urinary excretion of oxypurines. Therefore, ethanol-induced increases in the plasma concentration and urinary excretion of oxypurines seem to be ascribable to both ethanol-induced purine degradation and xanthine dehydrogenase inhibition. On the other hand, since an increase in the plasma concentration of purine bases correlated well with that of uridine in ethanol ingestion and fructose infusion (Fig 2), an increase in the plasma concentration of uridine by ethanol seems to reflect purine degradation. These results also suggested that the abrupt consumption of ATP induced by any substance besides ethanol and fructose (for example, xylitol infusion and severe exercise, etc.) in the body accelerates both purine and pyrimidine degradation, resulting in increases in plasma concentrations of both purine bases and uridine (Fig 1). In fact, xylitol infusion or severe exercise increased plasma concentrations of purine bases and uridine (our unpublished data, August 1996).

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