Effect of Glucagon on the Plasma Concentration of Uridine

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To determine whether glucagon affects the plasma concentration of uridine, we administered 100 mL physiological saline containing 1 mg glucagon or 100 mL physiological saline alone intravenously over 1 hour to healthy subjects. Glucagon decreased the plasma concentration of uridine from 5.72 ± 1.05 to 4.80 ± 0.60 μmol/L but increased the concentrations of cyclic adenosine monophosphate (cAMP) in plasma and pyruvic acid and lactic acid in blood 59-, 1.4-, and 1.3-fold, respectively. Although glucagon increased urinary excretion of uric acid, it did not affect the plasma concentration of purine bases (hypoxanthine, xanthine, and uric acid) or urinary excretion of oxypurines and uridine, indicating that glucagon does not affect purine degradation and suggesting that glucagon does not affect adenosine triphosphate (ATP) consumption–induced pyrimidine degradation. In contrast, physiological saline did not affect any of the measured variables. These results suggest that glucagon enhanced Na^+-dependent uridine uptake from the blood into the cells, since glucagon stimulates Na^+-dependent uridine uptake into cells in vitro.

THE MAIN PHYSIOLOGICAL function of glucagon is to regulate blood glucose together with insulin. For blood glucose regulation, glucagon promotes both glycogenolysis and gluconeogenesis and inhibits glycolysis in the liver. In addition, it promotes hepatocyte proliferation,[1] enterohepatic bile acid circulation,[2] and de novo purine synthesis[3] in the liver.

A recent study showed that glucagon stimulated Na^+-dependent uridine uptake in isolated rat liver in vitro.[4] Nucleoside transport pathways consist of Na^+-dependent and Na^+-independent types.[5-7] The former pathway was demonstrated in rat liver plasma membrane vesicles, with broad substrate specificity (K_m, 6 to 12 μmol/L for uridine and 8 to 14 μmol/L for adenosine).[5-8] Since the Na^+-dependent nucleoside transport pathway is expressed only in a few cell types with little endogenous nucleoside synthetic capacity (absorptive epithelia), its physiological function in liver cells is not clear. However, since these K_m values are in the range of the physiological concentrations,[9] it is suggested that the Na^+-dependent nucleoside transport pathway plays a role in nucleoside-induced modulation of several key enzymes in cells. In addition to glucagon-induced enhancement of Na^+-dependent nucleoside uptake,[4] a previous study[8] has demonstrated that Na^+-dependent nucleoside uptake is enhanced in the prereplicative phase soon after partial hepatectomy.

These findings suggest that this transport pathway is regulated by glucagon, which enhances Na^+-dependent uridine uptake after partial hepatectomy. Therefore, we investigated whether glucagon enhances uridine uptake from the blood into cells in humans.

SUBJECTS AND METHODS

Subjects and Protocol

Eleven men aged 34 to 47 years (body weight, 49 to 62 kg) participated in the study after provision of informed consent. The subjects had normal laboratory data. After an overnight fast except for water, the urine was completely voided, followed by collection of the 1-hour urine samples (first period). The first blood samples were drawn with heparinized syringes 30 minutes before the first urine collection. After the first urine samples were collected, 100 mL physiological saline containing 1 mg glucagon (glucagon infusion) was infused over 1 hour. The second urine samples were collected at the end of the infusion, and the second and third blood samples were drawn 30 minutes before and at the end of infusion. Two weeks later, a control study was performed with intravenous administration of only 100 mL physiological saline.

Blood and Urine Analyses

Plasma and urine concentrations of uridine, hypoxanthine, xanthine, and uridine were determined as described previously.[10] The uric acid concentration in plasma and urine was measured by the uricase method using a Hitachi 736 autoanalyzer (Tokyo, Japan). Lactic acid and pyruvic acid concentrations in blood were determined by enzymatic methods using a Determiner LA kit (Kyowa Medix, Tokyo, Japan) and a Determiner PA kit (Kyowa Medix), respectively. The plasma concentration of glucagon was measured by radioimmunoassay using a glucagon kit from Daiichi (Dacha RI; Tokyo, Japan), and plasma concentrations of cyclic adenosine monophosphate (cAMP) were also measured by radioimmunoassay, using an AMP kit (Yamasa Soy, Chiba, Japan).

Chemicals

Glucagon was purchased from Novo Nordisk (Copenhagen, Denmark). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Statistical Analysis

Values are expressed as the mean ± SD. The significance of differences between variables was analyzed using a two-tailed paired t test, except for the plasma glucagon level, which was analyzed by Wilcoxon's T test.

RESULTS

Effect of Glucagon on Plasma Concentrations of Glucagon and Glucose

Administration of glucagon increased plasma glucagon and glucose from 100 ± 13 pg/mL and 5.48 ± 0.03 mmol/L 30 minutes before beginning glucagon infusion to 13,263 ± 2,388 pg/mL ($P < .05$) and 8.94 ± 0.08 mmol/L ($P < .01$) 30 minutes after beginning glucagon infusion, respectively. In contrast,

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administration of physiological saline did not affect the plasma concentration of glucagon (99 ± 10 pg/mL 30 minutes before *v* 97 ± 8 pg/mL 30 minutes after beginning physiological saline infusion) or glucose (5.48 ± 0.54 mmol/L 30 minutes before *v* 5.40 ± 0.42 mmol/L 30 minutes after beginning physiological saline infusion).

Effect of Glucagon on Plasma Concentrations of Uridine and Purine Bases

Glucagon decreased the plasma concentration of uridine from 5.23 ± 0.99 μmol/L 30 minutes before beginning the glucagon infusion to 4.18 ± 0.65 μmol/L 30 minutes afterward and 3.64 ± 0.69 μmol/L 1 hour afterward (Fig 1), while physiological saline infusion did not affect the plasma concentration of uridine (5.20 ± 0.93 *v* 5.22 ± 0.89 *v* 5.20 ± 0.90 μmol/L). In contrast, glucagon or physiological saline did not affect plasma concentrations of purine bases (Table 1).

Effect of Glucagon on Urinary Excretion of Uridine and Purine Bases

Glucagon increased the urinary excretion of uric acid 1.7-fold but did not affect excretion of hypoxanthine and xanthine (Table 2), while physiological saline did not affect urinary excretion of uric acid, hypoxanthine, or xanthine (Table 2). In both studies (glucagon infusion and physiological saline infusion), urinary excretion of uridine was below detectable limits.

Effect of Glucagon on Concentrations of cAMP, Inorganic Phosphate in Plasma, and Pyruvic Acid and Lactic Acid in Blood

Glucagon increased cAMP concentrations in plasma 64-fold, pyruvic acid in blood 1.6-fold, and lactic acid in blood 1.5-fold and decreased plasma inorganic phosphate 0.7-fold, while physiological saline did not affect cAMP, pyruvic acid, lactic acid, or inorganic phosphate levels (Table 3).

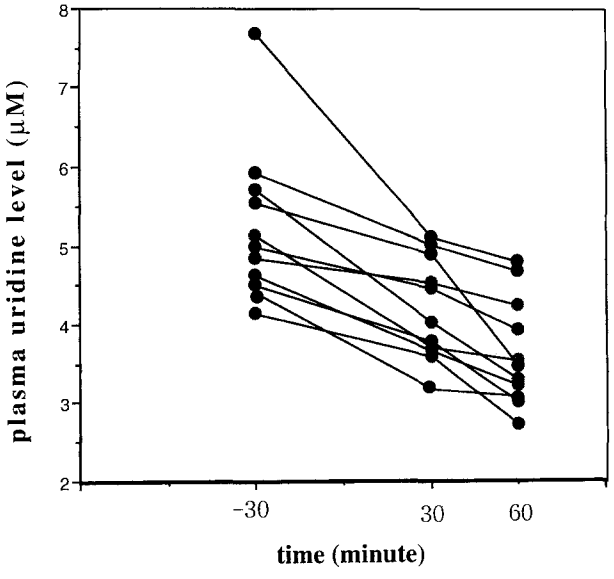

Fig 1. Effect of glucagon on plasma concentration of uridine 30 minutes before and 30 minutes and 1 hour after beginning glucagon infusion. *$P < .05$, **$P < .01$.

Table 1. Plasma Concentration (μmol/L) of Purine Bases (hypoxanthine, xanthine, and uric acid; N = 11)

Purine Base	−30 min	30 min	60 min
Glucagon infusion			
Hypoxanthine	2.08 ± 0.8	1.98 ± 0.83	1.88 ± 0.88
Xanthine	0.94 ± 0.34	0.84 ± 0.26	0.88 ± 0.35
Uric acid	333 ± 77	333 ± 83	333 ± 83
Physiological saline infusion			
Hypoxanthine	2.02 ± 0.73	2.00 ± 0.69	1.99 ± 0.67
Xanthine	0.94 ± 0.34	0.93 ± 0.31	0.93 ± 0.31
Uric acid	333 ± 71.4	333 ± 71.4	339 ± 71.4

NOTE. −30, 30, and 60 denote 30 minutes before and 30 minutes and 1 hour after beginning the glucagon infusion or physiological saline infusion.

DISCUSSION

In the present study, glucagon decreased the plasma concentration of uridine. Its mechanism is an acceleration of uridine uptake from the blood into the cells or a decreased release of uridine from the cells to the blood, because glucagon did not induce urinary excretion of uridine. In previous studies,[11-13] it has been shown that ischemia increases both purine and pyrimidine degradation because aerobic glycolysis is inhibited. In addition, we have demonstrated that the plasma concentration of uridine is increased with abrupt consumption of adenosine triphosphate (ATP) due to fructose infusion,[14] xylitol infusion,[15] ethanol ingestion,[14] or muscular exercise.[16] Namely, the consumption of ATP leads to increases in the concentration of adenosine diphosphate (ADP) and AMP. These changes in adenine nucleotides accelerate the conversion of AMP to inosine monophosphate, followed by purine degradation. Furthermore, since uridine diphosphate (UDP) is phosphorylated to uridine triphosphate using ATP, a decrease in the concentration of ATP leads to an increase in the concentration of UDP and uridine monophosphate (UMP), resulting in accelerated conversion of UMP to uridine. Since glucagon affects glycolysis,

Table 2. Urinary Excretion (μmol/h) of Purine Bases and Uridine (N = 11)

Parameter	1-Hour Excretion Before Infusion	1-Hour Excretion During Infusion
Glucagon infusion		
Hypoxanthine	6.28 ± 2.53	6.14 ± 1.90
Xanthine	4.88 ± 0.94	5.03 ± 1.17
Uric acid	178 ± 47	299 ± 83*
Uridine	ND	ND
Physiological saline infusion		
Hypoxanthine	5.66 ± 1.89	5.68 ± 2.01
Xanthine	4.81 ± 0.70	4.74 ± 0.65
Uric acid	174 ± 33	172 ± 33
Uridine	ND	ND

Abbreviation: ND, below the detection limit (0.2 μmol/h).
*$P < .01$.

Table 3. Concentration of cAMP, Inorganic Phosphate, Pyruvic Acid, and Lactic Acid (N = 11)

Parameter	−30 min	30 min
Glucagon infusion		
cAMP	7.9 ± 3.2	505.2 ± 130.9*
Pyruvic acid	0.069 ± 0.011	0.111 ± 0.039*
Lactic acid	0.84 ± 0.18	1.22 ± 0.34*
Inorganic phosphate	0.97 ± 0.13	0.71 ± 0.11*
Physiological saline infusion		
cAMP	8.7 ± 1.8	8.3 ± 1.6
Pyruvic acid	0.066 ± 0.007	0.066 ± 0.005
Lactic acid	0.81 ± 0.10	0.82 ± 0.09
Inorganic phosphate	1.00 ± 0.08	1.00 ± 0.09

NOTE. Inorganic phosphate, lactic acid, pyruvic acid, and cAMP are expressed as mmol/L, μmol/L, μmol/L, and pmol/mL.

*$P < .01$.

gluconeogenesis, and glycogenolysis related to ATP production, it may affect the plasma concentration of uridine along with the ATP consumption–related intracellular degradation of pyrimidine and purine. In fact, glucagon enhances purine degradation in patients with von Gierke's disease[17] and subjects on xylitol infusion.[15]

In the present study, glucagon increased blood glucose, pyruvic acid, and lactic acid, AMP, and urinary excretion of uric acid and decreased the plasma concentration of inorganic phosphate, as described in a recent study[18] showing that glucagon increased the urinary excretion of oxypurinol, uric acid, and xanthine in normal subjects on allopurinol, suggesting that glucagon affected the renal common transport pathway of uric acid, xanthine, and oxypurinol by stimulating the release of a liver-derived renal vasodilator. However, at the physiological level of purine bases, glucagon did not significantly affect the plasma concentration of purine bases or the urinary excretion of oxypurines (hypoxanthine and xanthine), indicating that glucagon did not significantly affect purine degradation. Therefore, it is suggested that the glucagon-induced decrease in the plasma concentration of uridine is not caused by a decrease in pyrimidine degradation, but by an increase in uridine uptake into the cells from the blood by glucagon, since glucagon stimulates Na^+-dependent uridine uptake in isolated rat liver in vitro.[4] This effect of glucagon on Na^+-dependent nucleoside transport is mimicked by dibutyryl cAMP, which is able to hyperpolarize the plasma membrane.[4] We recently demonstrated that dibutyryl cAMP decreased the plasma concentration of uridine in humans. In addition, the present study showed that glucagon increased the plasma concentration of cAMP, indicating that glucagon enhanced the production of cAMP in the liver and released it to the blood. These findings suggest that cAMP enhanced uridine uptake into the cells via the Na^+-dependent nucleoside transport pathway. It has been suggested that the physiological role of the Na^+-dependent nucleoside transport pathway is to preserve extracellular nucleoside for endogenous synthesis of nucleic acids,[7,8] since this nucleoside transport is enhanced in response to a mitogenic stimulus as in partial hepatectomy.[8] Therefore, the present study suggests that the enhancement of nucleoside transport by glucagon may clinically play a profitable role in nucleic acid biosynthesis in the physiological situation leading to hypertrophia and hyperplasia of the liver.

Although the effect of glucagon on the plasma concentration of uridine was pharmacological in the present study, the effect of glucagon on nucleoside transport may have physiological relevance, since the glucagon level in the portal vein is higher than in the peripheral vein and increases after partial hepatectomy in the prereplicative phase of liver growth, and further, the intake of amino acids decreased the plasma concentration of uridine together with the increased plasma glucagon level (T. Yamamoto, unpublished data, June 1997). However, the possibility that the direct effect of glucagon on pyrimidine metabolism may decrease the plasma concentration of uridine should not be neglected and requires study. Therefore, further examination including glucagon infusion at the plasma level in the portal vein induced by partial hepatectomy is needed.

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