Hepatic glycogen breakdown is implicated in the maintenance of plasma mannose concentration

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Submitted 24 September 2004; accepted in final form 5 November 2004

D-MANNOSE IS A MONOSACCHARIDE CONSTITUENT OF GYCOPROTEINS AND GLYCOLIPIDS. The concentration of free mannose in plasma ranges from 20 to 80 μM in healthy adults (7, 20, 22–24) but is elevated in patients with invasive candidiasis associated with immune deficiency (13, 21) and is decreased in patients with carbohydrate-deficient glycoprotein syndrome (2, 9, 16). In diabetic patients, the plasma concentrations of mannose are high, and plasma concentrations of mannose and glucose are positively correlated (19, 22, 24). In the past, determination of plasma mannose required special equipment and was time consuming, but in recent years, it can be achieved quickly and conveniently by HPLC (24) or an automated method using aldohexose dehydrogenase (22). With these techniques, the concentration of plasma mannose has been shown to decrease significantly 30–60 min after oral administration of glucose to healthy individuals (22, 24).

In mammalian cells, the donor GDP-mannose used for glycoprotein and glycolipid synthesis is derived through the pathway mannose 6-phosphate → mannose 1-phosphate → GDP-mannose. Mannose 6-phosphate is assumed to be formed by the following two pathways (5): 1) isomerization of intracellular fructose 6-phosphate by mannose-6-phosphate isomerase and 2) phosphorylation of mannose imported into the cell via a mannose-specific transporter. However, it is unknown how the plasma mannose necessary for the second pathway is supplied: negligible mannose is provided in the diet, and it has been generally accepted that no mannose production occurs through digestion of mannose-containing polysaccharides. Furthermore, no studies have examined tissue release of mannose. To make an advance in understanding the whole scheme of glycoprotein and glycolipid synthesis, we investigated here how plasma mannose is supplied.

METHODS

Materials. Nine-week-old Male Wistar rats and Goto-Kakizaki (GK) rats, a type 2 diabetes model, were obtained from Japan Clea (Tokyo, Japan). Rats were maintained on normal chow, using standard husbandry procedures. Animal care and procedures were approved by the Guiding Principles for the Care and Use of Laboratory Animals of Meijo University. 1,4-Dideoxy-1,4-imino-D-arabinitol (DAB), an inhibitor of glycopen phosphorylase, was synthesized according to the literature (15). Human insulin (Humarin R) was obtained from Eli Lilly (Indianapolis, IN).

Administration of agents. Two days before the experiment, rats underwent right atrial cannulation with silicon tubing (0.5 mm ID, 1.0 mm OD) via the jugular vein. All surgical procedures were performed under pentobarbital sodium anesthesia (50 mg/kg ip; Abbott Laboratories, Tokyo, Japan) and aseptic conditions. Aqueous solutions (200 μl) of epinephrine, insulin, sodium lactate, sodium lactate plus alanine, and DAB were administered via the cannula.

Glucose assay. Blood samples (150 μl) were collected from the cannula under unrestrained conditions every 5–15 min for 30–60 min and were immediately replaced with an equivalent volume of saline. Plasma glucose was assayed by the glucose oxidase method.

Insulin assay. Plasma samples were stored at −20°C until assayed. Insulin was measured by enzyme-linked immunosorbent assay using a commercial kit.

Plasma mannose assay. For mannose analysis, blood samples (90 μl) were diluted by addition of an equal volume of saline to which heparin (10 U/ml) had been added. After centrifugation, the superna-

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tants (120 μl) were mixed with 16 μl of 1.5 M perchloric acid. The supernatants obtained after centrifugation of the mixtures were used for the measurement of mannose. Mannose was assayed by the HPLC method, as described previously (24).

Liver perfusion. Liver perfusion was performed in the flow-through mode according to a published method (14). A medium (in mM: 2.8 or 5 glucose, 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, and 24.2 NaHCO₃, saturated with 95% O₂-5% CO₂, pH 7.4) heated at 37°C was perfused at a flow rate of 30 ml/min. When the effects of epinephrine and epinephrine plus chlorogenic acid (an inhibitor of glucose-6-phosphatase) were being ascertained, fed rats and a medium containing 5 mM glucose were used, and when the effects of lactate and lactate plus alanine were being ascertained, 24-h-fasted rats and a perfusate containing 2.8 mM glucose were used. After 10 min of preperfusion, a medium containing epinephrine, epinephrine plus chlorogenic acid, lactate, or lactate plus alanine was perfused. To measure the hepatic concentration of hexose 6-phosphates, after preperfusion for 10 min the flow rate was reduced to 21 ml/min, after which the right lobe was removed after ligation of its base and frozen in liquid nitrogen. After the liver had been perfused for 5 min more at 21 ml/min, perfusion was performed for 4 min more using a medium containing epinephrine. The left lobe was then removed and frozen in liquid nitrogen.

Fig. 1. Effect of oral administration of glucose on plasma concentrations of glucose (A and C), mannose (A and C), and insulin (B and D) in 12-h-fasted normal (A and B) and Goto-Kakizaki (GK) rats (C and D). Values are expressed as means (SD) of 6 (A and B) and 3 (C and D) determinations. *P < 0.05, **P < 0.01 vs. values at 0 min.

Fig. 2. Effect of intravenous administration of insulin on glucose (F) and mannose (E) concentrations in plasma of normal (A) and GK rats (B). Values are expressed as means (SD) of 4 (A) and 6 (B) determinations. *P < 0.05, **P < 0.01 vs. values at 0 min.
Perfusate glucose and mannose assays. The concentration of perfusate glucose was determined by the glucose oxidase method using a commercially available kit. The concentration of perfusate mannose was measured in the following manner. After 5 ml of each perfusate sample were lyophilized, the resulting lyophilized sample was dissolved in 500 µl of purified water and then centrifuged. To 400 µl of the supernatant, 100 µl of 1.5 M HClO₄ were added, and the resulting solution was centrifuged. The level of mannose in the supernatant obtained was measured by HPLC (24). For perfusate samples containing chlorogenic acid, the acid was removed before lyophilization by means of a Sep-Pak Plus Alumina A cartridge (Waters, Milford, MA) that had been rinsed with physiological saline, and the concentration of mannose was measured using the manner described above.

Assays of liver hexose 6-phosphates. Each frozen liver was homogenized using three volumes of 1.5 M HClO₄ and centrifuged. Subsequently, 200 µl of the resulting supernatant and 800 µl of 1 M Tris-HCl buffer (pH 8.0) containing 0.4 mM NADP were placed in a cuvette. Next, at a temperature of 37°C, 20 µl each of glucose-6-phosphate dehydrogenase (10 U), glucose-6-phosphate isomerase (5 U), and mannose-6-phosphate isomerase (38 U) were added in that order, and levels of glucose 6-phosphate, fructose 6-phosphate, and mannose 6-phosphate were determined by measuring absorbance at 340 nm.

Statistical analysis. The statistical analyses were performed by paired t-test with the level of significance set at P < 0.05.

RESULTS

Effect of glucose administration on plasma mannose concentration. When glucose was administered orally to 12-h-fasted normal rats, plasma mannose concentrations decreased significantly starting 20 min after plasma insulin concentration peaked (Fig. 1, A and B). The concentrations of plasma mannose and glucose in 12-h-fasted GK rats were significantly higher compared with those of 12-h-fasted normal rats (plasma mannose: 145.9 ± 2.4 and 100.3 ± 12.9 mM, respectively; plasma glucose: 14.6 ± 2.0 and 7.5 ± 0.8 mM, respectively). By oral administration of glucose to the GK rats, the concentration of plasma glucose increased, but the concentrations of plasma mannose and insulin did not change significantly (Fig. 1, C and D).

Effect of insulin administration on plasma mannose concentration. When insulin was administered intravenously to fed normal rats, the concentration of plasma mannose began to decrease significantly at 5 min after administration, to reach trough levels after 10 to 20 min (Fig. 2A). The concentration of plasma glucose recovered to preadministration levels 45 min after administration, and at that time the concentration of plasma mannose also returned to baseline levels. When insulin was administered intravenously to fed GK rats, it took longer for plasma mannose concentrations to decrease, and a significant decrease was seen at length 20 min after administration (Fig. 2B).

Effect of epinephrine administration on plasma mannose concentration. When epinephrine was administered intravenously to fed normal rats, concentrations of plasma mannose and glucose increased, and plasma mannose remained elevated for ≥30 min (Fig. 3A). Epinephrine administration did not affect the concentration of plasma insulin (data not shown). Intravenous administration of epinephrine to 48-h-fasted normal rats did not affect plasma mannose concentrations (Fig. 3B). Similarly, when DAB was administered intravenously to the fed rats 10 min before epinephrine was administered, plasma mannose concentrations did not increase (Fig. 3C).

Effects of epinephrine on mannose output and hepatic hexose 6-phosphates in perfused livers. Perfusion of a medium containing 5 mM glucose to the isolated livers of fed normal rats caused a constant hepatic release of mannose. Moreover,
when the perfusate contained epinephrine, significant increases in not only glucose output but also mannose output were evident (Fig. 4, A and B). However, in the presence of chlorogenic acid, epinephrine did not significantly increase either glucose output or mannose output (Fig. 4, C and D). Compared with preperfusion levels, hepatic levels of glucose 6-phosphate, fructose 6-phosphate, and mannose 6-phosphate in livers from normal rats were significantly greater 4 min after epinephrine perfusion (Fig. 5).

**Effect of gluconeogenic precursor administration on plasma mannose concentration.** Intravenous administration of either lactate alone or lactate plus alanine to 24-h-fasted rats significantly increased plasma glucose concentration starting from 10 min after administration, whereas plasma mannose concentration exhibited no marked changes (Fig. 6, A and B). When the isolated livers of 24-h-fasted rats were perfused using a medium containing sodium lactate, glucose output increased markedly, but mannose output decreased (Fig. 7).

**DISCUSSION**

The data in Fig. 1A demonstrating the decrease in plasma mannose concentration in contrast to the increase in plasma glucose concentration indicate that free glucose in plasma is not the direct source of plasma mannose. When glucose was administered orally, 12-h-fasted normal rats showed a decrease in plasma mannose concentration that occurred slightly later than the increase in plasma insulin, whereas 12-h-fasted GK rats exhibited no marked changes in the concentration of plasma insulin or mannose (Fig. 1, A–D), thus suggesting that insulin is involved in lowering the concentration of plasma mannose. In fact, insulin administration to fed normal rats caused a rapid fall in plasma mannose concentrations. However, when insulin was administered to fed GK rats, plasma mannose concentration decreased more slowly (Fig. 2, A and B). This lesser effect of insulin on the concentration of plasma mannose in GK rats may be related to hepatic insulin resistance (6, 17).

On the basis of Figs. 1 and 2, we hypothesized that reduced glycogen breakdown links the effect of insulin on the liver to the decreased concentration of plasma mannose, and we decided to investigate the changes in plasma mannose concentration that occurred on promoting hepatic glycogen breakdown. In fed normal rats, epinephrine increased the concentration of plasma mannose (Fig. 3A), but this effect was negated by 48 h of fasting (Fig. 3B), thus supporting our

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**Fig. 4.** Effect of epinephrine on output of glucose (A and C) and mannose (B and D) from perfused livers of chlorogenic acid-untreated (A and B) or -treated (C and D) rats. Values are expressed as means (SD) of 4 determinations. *P < 0.05, **P < 0.01 vs. values at basal conditions (from −3 to −2 min).
hypothesis. Additionally, when DAB (8, 12), a glycogen phosphorylase inhibitor, was administered, plasma mannose concentrations in fed normal rats did not increase in response to epinephrine (Fig. 3C), thus further supporting our hypothesis. The hypothesis that epinephrine increases the concentration of plasma mannose by accelerating hepatic glycogen breakdown was consistent with the finding that epinephrine increased mannose output from isolated rat livers (Fig. 4B). Furthermore, epinephrine did not increase mannose output from isolated rat livers in the presence of chlorogenic acid (10), a glucose-6-phosphatase inhibitor (Fig. 4D). This suggests that glucose-6-phosphatase is involved in the mannose output induced by glycogen breakdown. Recent reports have indicated that glucose-6-phosphatase in intact microsomes has the ability to hydrolyze mannose 6-phosphate as well as glucose 6-phosphate (1, 3, 18). Moreover, the present data demonstrate a significant increase in hepatic levels of glucose 6-phosphate, fructose 6-phosphate, and mannose 6-phosphate in response to epinephrine. Taken together, these findings strongly suggest that epinephrine stimulates glycogen breakdown to promote the formation of hexose 6-phosphates, including mannose 6-phosphate, and subsequently increases glucose-6-phospha-

Fig. 5. Effect of epinephrine perfusion on amount of hexose 6-phosphates in liver of normal rats. Open columns, hexose 6-phosphate contents measured before epinephrine perfusion; shaded columns, hexose 6-phosphate contents measured after epinephrine perfusion. G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; M6P, mannose 6-phosphate. Values are represented as means (SD) of 4 determinations. *P < 0.05, **P < 0.01 vs. preperfusion values.

Fig. 6. Effect of intravenous administration of sodium lactate (A) or sodium lactate plus alanine (B) on plasma concentrations of glucose (●) and mannose (○) in 24-h-fasted rats. Values are expressed as means (SD) of 4 experiments. *P < 0.05, **P < 0.01 vs. values at 0 min.

Fig. 7. Effect of sodium lactate on output of glucose and mannose from perfused liver of 24-h-fasted rats. Values are represented as means (SD) of 4 determinations. *P < 0.05, **P < 0.01 vs. values at basal conditions (from −2 to 0 min).
High-Tech Research Center Project from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Acknowledgments

We are grateful to Omiko Sugiura for administrative help.

Grants

This work was supported, in part, by a grant-in-aid (To I. Miwa and T. Taguchi) for the Scientific Frontier Research Project from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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