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Decreased Glucose Production from Maltose in Perfused Kidney of Streptozotocin Diabetic Rats (42412)

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Abstract. To elucidate the role of renal maltase in the metabolism of circulating maltose, glucose production from maltose was investigated in perfused kidney of normal and streptozotocin (STZ) diabetic rats. The kidney was perfused with 150 mg/dl maltose for 30 min and perfusate maltose and glucose were measured. Perfusate glucose concentration in the kidney of control rats gradually increased during perfusion. That in the kidney of diabetic rats was significantly lower than that in controls. Perfusate maltose concentration in the kidney of diabetic rats was significantly higher than that in controls. These results suggest that circulating maltose may enter kidney cells and subsequently metabolized to glucose, and that maltose uptake and glucose production were decreased in the kidney of STZ diabetic rats.

Weser and Sleisenger (1) have reported that circulating maltose may be hydralyzed by extraintestinal maltases and subsequently metabolize in man and rats. Young and Weser (2) suggested that slowly increased serum glucose levels after injection of [U-^{14}C]maltose represent reentry of labeled glucose from tissue sources. Maltase (α-D-glucoside glucohydrolase) is the major dissacharidase in rat renal brush border membranes (3, 4), and maltase activity in renal cortex homogenates is reported to be changed in aging rats and streptozotocin (STZ) diabetic rats (5–7). However, the role of renal maltase in the metabolism of circulating maltose is still unknown. To elucidate this point, we investigated the metabolism of maltose in perfused kidney of normal and STZ diabetic rats.

Materials and Methods. *Materials.* Dextran T-70 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Bovine serum albumin (BSA, fraction V) and maltase (α-D-glucoside glucohydrolase, EC 3.2.1.20) were obtained from Sigma Chemical (St. Louis, Mo.). Maltose monohydrate was purchased from Wako Chemical (Osaka). STZ was obtained from Upjohn Co. (Kalamazoo, Mich.).

Animals and induction of diabetes. Male Wistar albino rats weighing about 100 g were used in the present study. The rats were divided into two groups, control and experimental. The rats were fasted for 18 hr with free access to water. The experimental rats were injected with 50 mg/kg STZ ip (freshly dissolved in 0.05 *M* citrate buffer, pH 4.5), while the control rats were injected with vehicle alone. All rats were then allowed free access to laboratory chow. One-half of the diabetic rats were administered insulin (Lente insulin, 6 U/day) sc beginning 4 days after the induction of diabetes and continuing for 10 days. Two weeks later, the diabetic rats with or without insulin treatment were used in the following studies, together with control rats.

Perfusion of kidney. The technique for the isolated, perfused rat kidney was a modification (8, 9) of the method of Nishiitsutsuji-Uwo *et al.* (10).

The abdomen was opened through a midline incision, and the intestines were placed to the animal's left. The thin strands of connective tissue between the right lobe of the liver and the vena cava were cut and a loose ligature was placed around the inferior vena cava above and below the right renal vein, and around the right renal artery. The inflow cannula was inserted through the superior mesenteric artery into the right renal artery. The ligature around the inferior vena cava above the right renal vein was then tied, and an outflow cannula was inserted into the vena cava. Then the kidney was isolated and perfused without recirculation at flow rate of 4 ml/min.

Perfusion method. The perfusion medium

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consisted of Krebs–Ringer bicarbonate buffer containing 0.5% BSA and 4.6% Dextran T-70. The kidney was perfused with the medium not containing maltose for 15 min, and then was perfused with the perfusate containing 150 mg/dl maltose for 30 min. The venous effluent was collected every 5 min, and stored at −20°C until the time of measurement. During perfusion, the medium and the chamber were warmed and kept at 37°C and the medium was bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was maintained at 7.4.

Maltose and glucose determination. Blood glucose and perfusate glucose were measured by glucose oxidase method. Perfusate maltose was determined by incubating 0.1 ml of perfusate with 1 U of commercial maltase dissolved in 0.2 ml of 0.05 *M* sodium citrate for 90 min at 37°C. After incubation, the net increase in glucose was measured by the glucose oxidase method. Perfusate maltose concentration was calculated by the following formula: glucose concentration after incubation − glucose concentration before incubation.

Statistical evaluation of data. Analysis of variance and Student's *t* test were carried out using standard statistical procedures. The data are presented as the means ± SD.

Results. *Body weight and blood glucose level.* Body weights and blood glucose levels were 196 ± 10 g and 187 ± 10 mg/dl, 155 ± 7 g and 650 ± 150 mg/dl, and 173 ± 10 g and 320 ± 80 mg/dl in control, diabetic, and insulin-treated diabetic rats, respectively.

Maltose and glucose concentration in the perfusate. As shown in Fig. 1, perfusate maltose concentration in the kidney of control rats was gradually decreased during perfusion. That in the kidney of diabetic rats was significantly higher than that in controls, while that of insulin-treated diabetic rats was similar to controls.

Perfusate glucose concentration in the kidney of control rats gradually increased during perfusion. That in the kidney of diabetic rats was significantly lower than that in controls. That in the kidney of insulin-treated diabetic rats was significantly higher than that of diabetic rats but significantly lower than that of control rats.

Discussion. The production of glucose from maltose is determined by both the uptake of maltose and the activity of maltase in the tissues. An advantage of the perfused kidney preparation is that the contributions of both maltose uptake and maltase activity to glucose production can be assessed simultaneously. The present study clearly demonstrated that circulating maltose was metabolized to glucose by the kidney, and the glucose appeared in the renal vein. These results agreed with the report of Young and Weser (2) that increase of serum glucose after injection of maltose represents reentry of glucose from tissue sources. As human kidney tissue does have maltase activity, an increase of serum glucose after maltose injection may partly be due to reentry of glucose from the kidney. Some of the maltose is filtered at the glomerulus, and then hydrolyzed to glucose by brush border maltase (11). This glucose may be reabsorbed by the tubular cell. The concentration of glucose in the perfusate may be due in part to glucose released by maltose hydrolysis, and maltose is possibly picked up by other kidney cells which contain an acid maltase and used for fuel via hydrolysis.

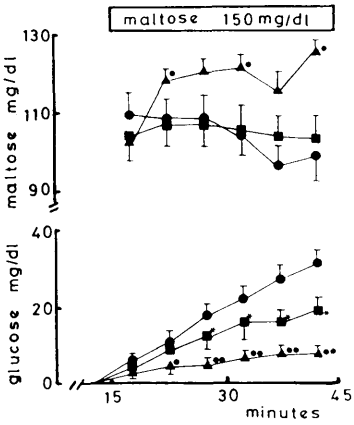

FIG. 1. Maltose and glucose concentration in the perfusate. The bars represent SD. ●, control (n = 6); ▲, diabetic (n = 6); ■, insulin-treated diabetic (n = 6). $^{\bullet}P < 0.05$; $^{\bullet\bullet}P < 0.02$, significantly different from control. $^{*}P < 0.05$, significantly different from control and diabetic.

Glucose production from maltose by the perfused kidney of STZ diabetic rats was significantly lower than that of controls. Sternberg and Spiro (6) have reported that an increased activity of maltase was observed in kidney cortex homogenates and supernatants of STZ diabetic rats. Perfusate maltose concentration was significantly higher in the kid-

ney of diabetic rats than in controls. This suggests that maltose uptake by the kidney of diabetic rats was significantly lower than that of controls. The decreased glucose production from maltose in diabetic kidney may be due to the reduction of renal maltose uptake. Permeability of maltose to the kidney cell may be reduced in diabetic states. Since Young and Weser (12) have reported that urinary recovery of ^{14}C after the intravenous administration of [^{14}C]maltose was similar in normal and diabetic subjects, urinary excretion of maltose was not investigated in the present study. However Young *et al.* (13) reported that insulin administration in normal subjects increased the reabsorption Tm for filtered maltose–glucose (decreased urine excretion) when maltose was intravenously infused at the slow infusion rate. In diabetic rats, low glucose concentration in the perfusate may be accounted for by increased urine excretion of maltose–glucose. And turnover and/or flux rates of maltose may be altered during perfusion. Further studies are needed to clarify these points.

The decreased glucose production may not be due to the renal toxicity of STZ because glucose production from perfused kidney was increased by insulin treatment. In the present study, neither maltose nor glucose in the perfusate reached a steady state. Maltose may be metabolized gradually by the kidney *in vitro*.

In summary, we conclude that circulating maltose may enter kidney cells and subsequently be metabolized to glucose, and that decreased glucose production from maltose in the kidney of STZ diabetic rats may be due to a reduction of maltose uptake in the tissue.

1. Weser E, Sleisenger MH. Metabolism of circulating disaccharides in man and the rat. J Clin Invest **46**: 499–505, 1967.
2. Young SJM, Weser E. The metabolism of circulating maltose in man. J Clin Invest **50**:986–991, 1971.
3. Sacktor B. Trahelase and the transport of glucose in the mammalian kidney and intestine. Proc Natl Acad Sci USA **60**:1007–1014, 1968.
4. Berger SJ, Sacktor B. Isolation and biochemical characterization of brush border from rabbit kidney. J Cell Biol **47**:637–645, 1970.
5. Reiss U, Sacktor B. Alteration of kidney brush border membrane maltase in aging rats. Biochim Biophys Acta **704**:422–426, 1982.
6. Sternberg M, Spiro RG. Studies on the catabolism of the hydroxylysine-linked disaccharide units of basement membranes and collagens: Isolation and characterization of a rat kidney α-glucosidase of high specificity. J Biol Chem **254**:10329–10336, 1979.
7. Sternberg M, Andre J, Peyroux J. Inhibition of the α-glucosidase specific for collagen disaccharide units in diabetic rat kidney by in vivo glucose levels: possible contribution to basement membrane thickening. Diabetologia **24**:286–289, 1983.
8. Ikeda T, Ito Y, Murakami I, Mokuda O, Kuno S, Tokumori Y, Tominaga M, Mashiba H. Effect of diabetes on triiodothyronine and reverse triiodothyronine production in perfused rat liver and kidney. Diabetes **34**:647–652, 1985.
9. Ikeda, T., Honda M, Murakami I, Kuno S, Mokuda O, Tokumori Y, Tominaga M, Mashiba H. Effect of TSH on conversion of T_4 to T_3 in perfused rat kidney. Metabolism **34**:1057–1060, 1985.
10. Nishiitsutsuji-Uwo JM, Ross BD, Krebs HA. Metabolic activities of the isolated perfused rat kidney. Biochem J **103**:852–862, 1967.
11. Silverman M. Brush border disaccharidases in dog kidney and their spatial relationship to glucose transport receptors. J Clin Invest **52**:2486–2494, 1973.
12. Young EA, Weser E. The metabolism of maltose after intravenous injection in normal and diabetic subjects. J Clin Endocrinol Metab **38**:181–188, 1974.
13. Young EA, Drummond A, Cool DA, Cioletti LA, Crain M, Traylor JB, Weser E. The effect of insulin on the metabolism of parenteral maltose in man. J Clin Endocrinol Metab **50**:764–772, 1980.

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