ELSEVIER

Biochimica et Biophysica Acta 1291 (1996) 89–95

BBA Biochimica et Biophysica Acta

Common reabsorption system of 1,5-anhydro-D-glucitol, fructose, and mannose in rat renal tubule

Toshikazu Yamanouchi *, Takaomi Shinohara, Nobuyuki Ogata, Yumi Tachibana, Ieo Akaoka, Hideo Miyashita

Second Department of Internal Medicine, University of Teikyo, Itabashi-ku, Tokyo 173, Japan

Received 22 April 1996; accepted 13 May 1996

Abstract

1,5-Anhydro-D-glucitol (AG) is a major polyol, 99.9% of which is reabsorbed by the kidney. However, such reabsorption is inhibited by competition with glucose excreted in excess, i.e., glucosuria. Under such conditions, AG is excreted into the urine. We administered various types of sugars to rats by continuous intravenous infusion for two hours to evaluate the competition between AG and these sugars for renal reabsorption in vivo. The reabsorption of AG was significantly inhibited by competition with fructose and mannose. The excretion of AG in the 120 min after a load of 3.64 mmol of fructose was 1.99 ± 0.33 μmol, that after 3.64 mmol of mannose loading was 2.34 ± 0.43 μmol. These levels were comparable to the AG excretion observed after the administration of the same amount of glucose (3.87 ± 0.61 μmol). No competition was observed with sucrose, xylose, myoinositol or galactose. The reabsorption of fructose and mannose was significantly inhibited by the presence of AG ($P < 0.001$) after a mixed load. Results suggest that AG is reabsorbed in the renal tubule by an AG/fructose/mannose-common transport system that is distinct from the major glucose reabsorption system. These findings may help to clarify the specific transport systems for various sugars in the renal tubule, as well as their physiological importance.

Keywords: 1,5-Anhydroglucitol; Fructose; Mannose; Sugar transport; Renal tubule

1. Introduction

1,5-Anhydroglucitol (AG), which is a six-carbon chain monosaccharide in 1-deoxy form glucopyranose, is one of the most abundant polyols in the human body [1,2]. The serum concentration of AG is reduced in patients with diabetes, which provides a sensitive indicator of glycemic control [3–5]. AG originates mainly in the diet. It is well absorbed from the intestine, and is present in many tissues [6]. It is actively reabsorbed by the renal tubule and is excreted into the urine when its serum concentration exceeds the renal threshold for reabsorption [2,7,8]. Although its kinetics in the human body have recently been described, little is known about its metabolic pathway and cellular kinetics.

The structural similarities AG and glucose suggests that AG uptake is carried out by glucose transporters. Recently, however, it was suggested that rat hepatoma cells [9] and human polymorphonuclear leukocytes [10] possess an AG-specific carrier distinct from the glucose transporters. We previously reported that the human erythroleukemia cell line K-562 possesses a facilitated transport system, with a high K_m of 127 mmol/l, that is not affected by glucose, fructose, galactose, mannose, or fucose [11]. Physiological studies and clinical observations suggest that transport systems in the kidney and intestine differ from these transporters. Virtually all AG is absorbed in the intestine of hamsters [12,13] and humans [6] through an energy-requiring process, in competition with glucose. About 99.9% of the AG is absorbed by the renal tubules in rats [8] and humans [6]. Such reabsorption competes with that of glucose in the presence of hyperglycemia [2,4]. Whether the absorption of this polyol is mediated by glucose transporters [14–16], a different transporter, or both is not known. We found a specific inhibition of AG reabsorption in the absence of glucosuria in patients receiving long-term hyperalimentation, which suggests the existence of a carrier system that is selective for AG [17]. Little is known about the relationship between AG and

* Corresponding author. Fax: +81 3 53751178.

PII S0304-4165(96)00050-5

renal mannose and/or myoinositol transporters. We therefore evaluated the interaction between AG and various renal sugar transporters in the rat in vivo.

2. Materials and methods

2.1. Materials

AG was obtained from Nippon Kayaku Co., Ltd. (Tokyo). D-Glucose, D-fructose, D-mannose, D-galactose, D-xylose, myoinositol and sucrose were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 1,5-Anhydro-D-mannitol (AM) was purchased from Sigma Chemical (St. Louis, MO, USA), and pyranose oxidase (PROD) was obtained from Takara Shuzo Co., Ltd. (Kyoto, Japan). [U-^{13}C]AG was a generous gift from Dr. H. Akanuma, University of Tokyo, Tokyo.

2.2. Infusion of various sugars

Male Wistar rats, 300–350 g, were allocated to two groups. In the first group, rats were separated into eight subgroups (each subgroup comprised 5–12 rats) and infused continuously for 120 min intravenously with one sugar: AG, 0.30, 0.61 or 1.73 M; glucose, 3.48 M; fructose, 3.48 M; mannose, 3.48 M; myoinositol, 3.48 M; galactose, 3.48 M; xylose, 4.15 M; or sucrose, 1.83 M; or control saline. In the second group, rats were separated into three subgroups (each subgroup comprised 7–12 rats) and infused intravenously with various concentrations of fructose, 2.91–7.28 M, mannose, 2.91–7.28 M; or AG, 0.26–2.18 M. In the third group, rats were separated into two subgroups (each subgroup comprised 18 rats) and infused intravenously with various concentrations of two mixed sugars, viz., AG, 0.60–1.73 M, and fructose, 0.60–3.48 M; or AG, 0.60-1.73 M, and mannose, 0.60–3.48 M.

Rats were anesthetized (pentobarbital sodium, 50 mg/kg, i.p.) after being deprived of food for 24 h. Blood was sampled from the jugular vein at specific intervals. Plasma concentrations of glucose, AG, and the various sugars were measured at specified intervals for up to 120 min. Each test sugar was dissolved in sterile distilled water and injected intravenously for the first 5 min ($\sim$ 0.75 ml/kg), followed by continuous intravenous infusion [2]. The continuous infusion (rate $\sim$ 20 μl/kg body wt^{-1}/min^{-1}) was performed for up to 120 min with a PSW-31 micro-infusion pump (Nikkiso, Tokyo). The total amount of each sugar administered was as follows: xylose, 4.36 mmol/2 h; sucrose, 1.92 mmol/2 h; glucose, fructose, mannose, myoinositol, or galactose, 3.64 mmol/2 h; and AG, 1.82 mmol/2 h. For AG, we used an amount one-half that of the other sugars because of its expense and limited supply. Urine was collected from the bladder 2 h after the infusion of each sugar. Any rats suspected of suffering from hemodynamic insufficiency based on the urinary excretion of creatinine $\leq$ 40 μM were excluded from the study.

2.3. Assay

Plasma glucose concentration was measured with a Fuji Dri-Chem 2000 analyzer system (Fuji Photo, Tokyo). Quantitative measurement of urinary glucose concentration was performed by the aldose 1-epimerase-glucose oxidase method [18], which can detect concentrations of urinary glucose as low as 0.03 mM. The urinary creatinine level was determined by a colorimetric assay kit (Iatron Lab. Inc., Tokyo).

Plasma AG concentration was determined by gas-liquid chromatography (GLC) according to a modified method reported previously [6,11]. In brief, plasma (200 μl) was added to an internal standard, 10 μl of AM (1 mM), and mixed with 15 μl of 60% trichloroacetic acid (TCA) (Sigma). Following centrifugation, the supernatant was applied to a two-layer column containing, from the bottom, the H-form (0.3 ml) of the cation exchanger AG50W × 8 (Bio-Rad Laboratories, Richmond, CA) and the OH-form (0.5 ml) of the anion exchanger AG1 × 8, and the eluate was evaporated. Samples with high concentrations of AG were diluted 1:30 to 1:50 with saline. The residue was peracetylated for gas chromatography analysis on a gas-liquid chromatograph (Model GC-14A, Shimadzu, Kyoto) fitted with a fused-silica capillary column (HiCap-CBP1, Shimadzu, Kyoto). Isothermic chromatography was performed at 180°C for 18 min, followed by column cleaning at 220°C for 10 min. For these plasma samples, the interassay coefficient of variation (CV) was 2.0%, which the intra-assay CV was 1.4%.

The urinary concentration of AG was determined by gas chromatography/mass spectrometry (GC/MS), according to a method reported previously [6]. Preparation of urine for the microassay of AG required an additional borate-form exchanger column. AG was measured by gas-liquid chromatography combined with mass spectrometry, using [U-^{13}C]AG as the internal standard [19]. First, 0.59 nmol (100 ng) of [U-^{13}C]AG was added to the urine sample (200 μl). After treatment with TCA, the supernatant was applied to a three-layer column containing (from the bottom) the H-form of a cation exchanger and the OH and borate forms (0.15 ml) of an anion exchanger (AG1 × 8), and the eluate was evaporated. When a crystalline residue had been obtained, it was chromatographed on a Sephadex G-25 (Pharmacia, Uppsala, Sweden) column. Samples with high concentrations of AG were diluted 1:20 to 1:50 with distilled water. The resulting residue was acetylated for GC/MS analysis by a selected ion monitoring method in which $m/z = 170$ and 175 represented AG and [U-^{13}C]AG, respectively. The lower limit of detection of AG was 0.2 μM [6]. For these urine samples, the interassay CV was 8.0%; the intra-assay CV was 5.9%.

The plasma concentration of test sugar loaded was

determined from samples diluted 1:10 with saline. Diluted plasma (200 μl) was added to an internal standard, 5 μl of AM (30 mM), and treated with TCA. To remove glucose, the supernatant was evaporated, then mixed with pyranose oxidase (PROD) solution and incubated at 37°C for 60 min. 2.38 U of PROD in 1 ml of 0.0667 M phosphate buffer was pre-incubated at 37°C for 30 min and supplied as PROD solution. After evaporation, the residue was peracetylated for GLC. PROD treatment was used because, in the presence of a substrate concentration of ~ 10 mM, its relative response for fructose, myoinositol, mannose and galactose was 0%, 0%, 0.7 ± 0.3% and 1.4 ± 0.4% that of glucose, respectively. The activity of PROD is greatly influenced by its purity and the concentration of the substrate [20]. PROD treatment was not used in the determination of sucrose and xylose concentrations because the separation and identification of these sugars were not influenced by a large amount of glucose on GLC. Isothermic chromatography was performed at 180°C for xylose, 200°C for fructose, mannose, myoinositol, and galactose, and 300°C for sucrose.

Urine samples were diluted 1:20 with distilled water. A volume of 200 μl of each urine sample added to 20 μl of AM (30 mM) was used in measuring the concentrations of various sugars. The remainder of the procedures used for urinalysis was similar to that used for plasma.

2.4. Statistical analysis

Data are reported as mean ± S.E. The significance of the differences between groups was calculated by Student's *t*-test. Differences between groups were estimated by Scheffe's test for single subgroups. The distribution of the excretion of the loaded sugars was compared by the chi-square test and the analysis of variation (ANOVA). A value of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Plasma and urinary concentrations of AG and test sugars after various sugar loads

The plasma concentration of each sugar showed a square-wave increment after its initial large, acute injection followed by a plateau that was maintained for 120 min by administering the continuous intravenous infusion (Fig. 1). The plasma concentration of each test sugar was maintained at about the level shown in Table 1. As calculated from urinary excretion of each loaded sugar during the 120-min infusion, renal retention was high for fructose and mannose, low for sucrose, and intermediate for AG, galactose, myoinositol, and xylose (Table 1).

Plasma concentration of AG and glucose did not change during the infusion of control saline or galactose, and no urinary excretion of glucose was observed in these two

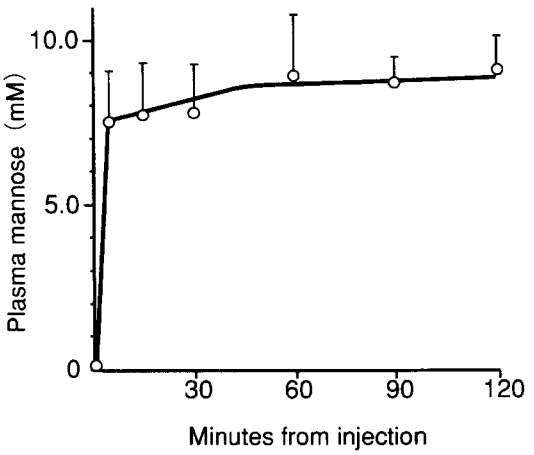

Fig. 1. Time course of plasma concentration of mannose after an initial bolus injection of mannose followed by its continuous intravenous infusion for 120 min. This pattern is representative of the plasma concentration of each of the sugars loaded in these experiments. Values are means ± S.E.

groups (Fig. 2A,B). Similar results were obtained in the myoinositol-loaded group (data not shown). The Plasma level of AG showed no significant change following the sucrose-load. Although the plasma concentration of glucose was slightly increased at the final time point, the urinary excretion of glucose was negligible (Fig. 2C). This finding shows that the slight hyperglycemia persisted for only a short period before the end of the experiment. Xylose loading significantly increased the plasma level of glucose. Glucosuria (6.0 ± 2.1 μmol) was accompanied by a significant reduction of plasma AG (Fig. 2D). In contrast, loading with fructose or mannose significantly reduced the plasma level of AG (Fig. 2E,F). Both groups exhibited slight terminal hyperglycemia, but the urinary concentration of glucose was negligible, as was observed with the sucrose load.

Table 2 shows the percent decrease in the plasma AG concentration and the amount of urinary AG during the 120 min infusion of each sugar. There were no significant differences in the rate of reduction of plasma AG or the

Table 1
Total dose, plasma concentration, and urinary recovery of test sugar, after intravenous injection

Sugar	Dose (mmol)	Plasma concentration (mM)	Urinary recovery (%)
Xylose	4.36	11.7 ± 1.0	23.1 ± 4.9
Sucrose	1.92	4.6 ± 0.7	44.7 ± 8.5
Myoinositol	3.64	8.3 ± 1.3	21.9 ± 6.2
Galactose	3.64	8.1 ± 1.1	27.4 ± 6.8
Fructose	3.64	9.9 ± 1.0	7.1 ± 0.8
Mannose	3.64	9.0 ± 1.4	9.5 ± 1.1
Glucose	3.64	24.1 ± 2.3	23.4 ± 7.1
AG	1.82	7.3 ± 0.6	26.7 ± 2.0

Values are means ± S.E. Five to twelve rats were tested in each subgroup.

amount of urinary AG among the galactose-loaded, myoinositol-loaded, sucrose-loaded, or control (saline) groups. There is a rapid efflux of free AG from the tissues into the blood. Equilibrium is quickly achieved under conditions of a low AG concentration. Thus, one can estimate AG depletion from the change in its plasma concentration [4,6]. If AG was metabolized in this period, its amount excreted in urine would differ from the calculated loss. The depletion of AG (μmol) was calculated to be 0.59 ± 0.13, xylose; 2.08 ± 0.29, fructose; 2.25 ± 0.39, mannose; and 3.61 ± 0.44 glucose, respectively. These values did not differ significantly from the amounts excreted in urine (Table 2). Thus, the reduced plasma concentration of AG in these groups appeared to be due primarily to urinary excretion.

The urinary AG/urinary loaded-sugar ratios, using

Table 2
Decrease in level of plasma AG and amount of AG excreted in urine during 120-min infusion of various sugars or saline

Compound	Decrease in plasma level of AG (%)	Urinary excretion of AG (μmol/2 h)
Saline	1.1 ± 0.3	0.01 ± 0.01
Sucrose	1.6 ± 0.4	0.03 ± 0.02
Myoinositol	1.9 ± 0.3	0.03 ± 0.01
Galactose	4.2 ± 1.5	0.05 ± 0.01
Xylose	16.8 ± 3.8 *	0.63 ± 0.11 *
Fructose	41.2 ± 4.3 **	1.99 ± 0.33 **
Mannose	58.6 ± 4.3 **	2.34 ± 0.43 **
Glucose	78.2 ± 5.7 **	3.87 ± 0.61 **

Samples with low concentrations of AG were concentrated by evaporation and then measured. Values are means ± S.E. * Significantly different from saline-loaded group, $P < 0.01$. ** Significantly different from saline-loaded group, $P < 0.001$.

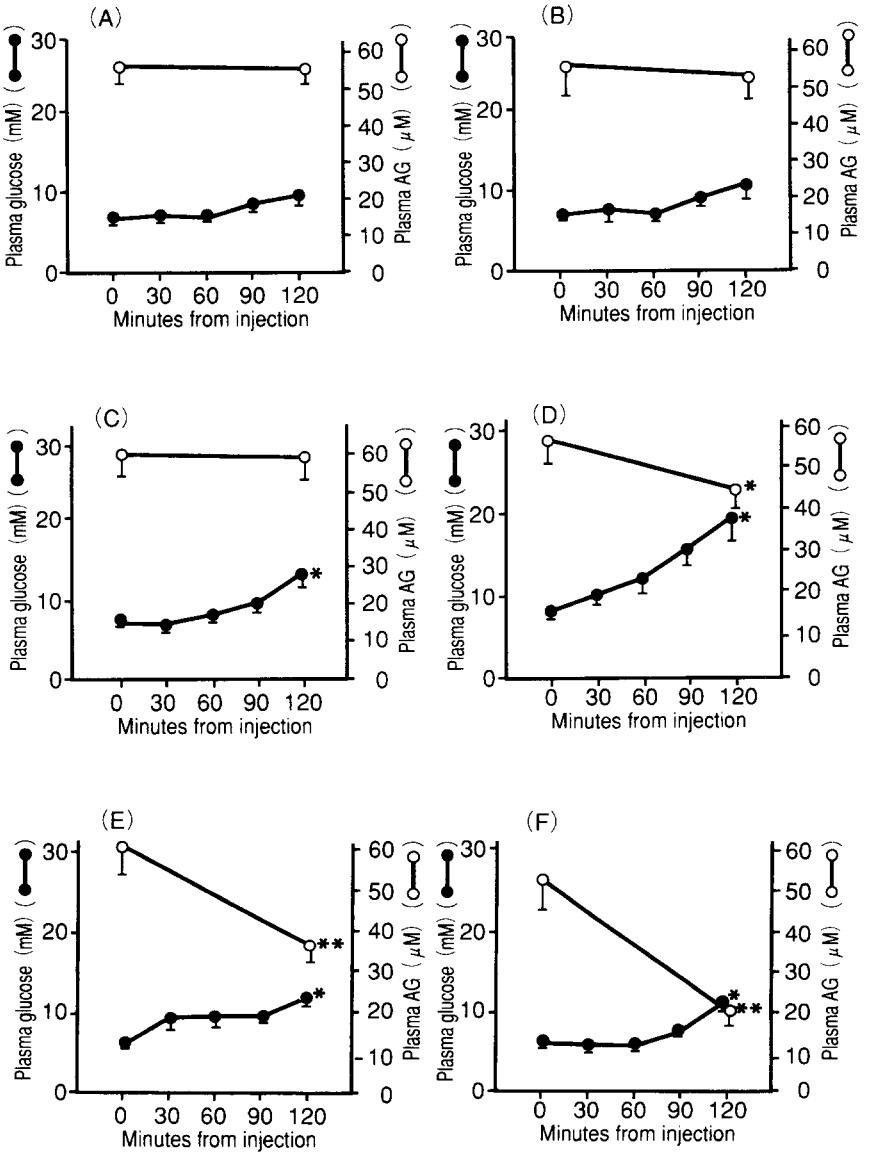

Fig. 2. Time course of plasma concentration of glucose and of AG following loading. (A) Saline control; (B) galactose; (C) sucrose; (D) xylose; (E) fructose; (F) mannose. * $P < 0.01$, ** $P < 0.005$ compared with plasma concentration of glucose or AG before the injection of the test sugar. The time course in the myoinositol experiment resembled that of galactose and is thus not shown.

Table 3
Urinary 1,5-anhydro-D-glucitol/urinary loaded-sugar ratio

Test sugar	Urinary AG/test sugar ratio (mean value)
Galactose	0.00005 *
Myoinositol	0.00004 *
Sucrose	0.00003 *
Xylose	0.0006 * *
Fructose	0.008
Mannose	0.007
Glucose	0.005

Urinary AG/test sugar ratio = urinary AG concentration (M)/urinary loaded-sugar concentration (M).
* Significantly different from glucose loaded group, $P < 0.0001$. * * Significantly different from glucose-loaded group, $P < 0.001$.

0.005 for glucose as the baseline, yielded values for galactose, myoinositol, sucrose, and xylose that were lower by two or three orders of magnitude. Values for fructose and mannose were essentially equal to that obtained for glucose (Table 3).

3.2. Competition of fructose and mannose for AG reabsorption

When we varied the dose of fructose or mannose, the urinary excretion of AG was strongly correlated with that

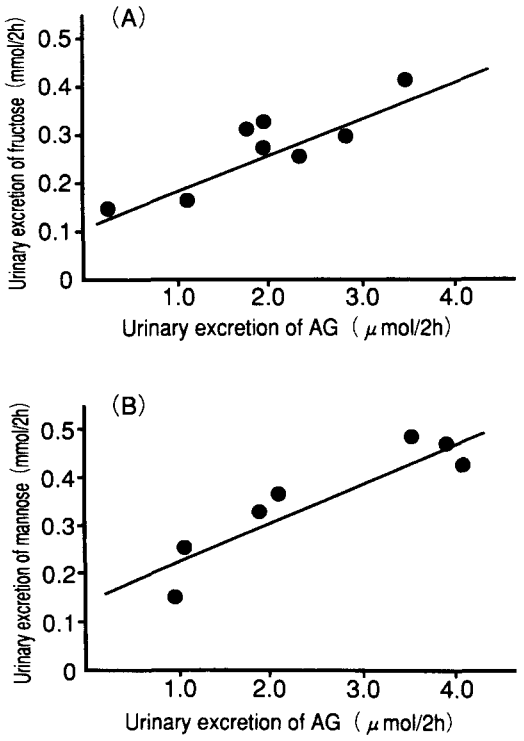

Fig. 3. (A) Correlation between urinary excretion of fructose and AG during the infusion of various concentrations (2.91–7.28 M) of fructose for 120 min. $y = 0.07x + 0.11$, $r = 0.865$, $P < 0.001$. (B) Correlation between the urinary excretion of mannose and AG during the infusion of various concentrations (2.91–7.28 M) of mannose for 120 min. $y = 0.08x + 0.14$, $r = 0.911$, $P < 0.001$.

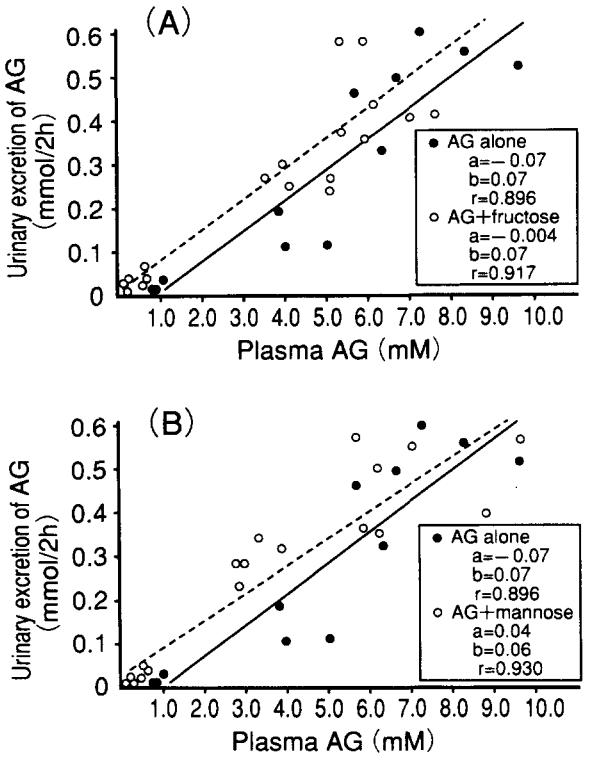

Fig. 4. Effect of fructose (A) and mannose (B) on excretion of AG. Fructose and mannose were each infused at a concentration equal to or slightly higher than that of AG (molar ratio: 1.0–5.0). Linear regression analysis revealed a positive correlation between the urinary excretion of AG and the plasma concentration of AG in the AG-alone control group, the AG plus fructose group, and the AG plus mannose group. When the slopes of the linear regressions generated from each group of rats (b in the equation, $y = a + bx$) were compared (AG alone, AG plus fructose, and AG plus mannose), no significant differences were noted in values for a and b.

of either fructose or mannose, respectively (Fig. 3A,B). An increase in mannose excretion was positively correlated with an increase in the dose of fructose ($r = 0.837$, $P < 0.001$). A highly positive correlation was observed between the amount of fructose excreted and the dose of mannose ($r = 0.811$, $P < 0.001$). To elucidate the relative affinity of the common transporter for AG, fructose, and mannose, we observed the competitive inhibition of reabsorption of each sugar by the other test sugar that was infused at a concentration equal to, or slightly higher than, that of the former (molar ratio 1.0–5.0). A dose of fructose or mannose similar to that of AG did not significantly affect the relationship between the plasma concentration and the urinary excretion of AG. Neither sugar promoted the urinary excretion of AG (Fig. 4A,B). In contrast, a similar level of AG showed a significant influence on the relationship between the plasma concentration and the urinary excretion of each fructose and mannose, and induced a significantly greater excretion of fructose and mannose than was observed with either sugar alone (Fig. 5A,B).

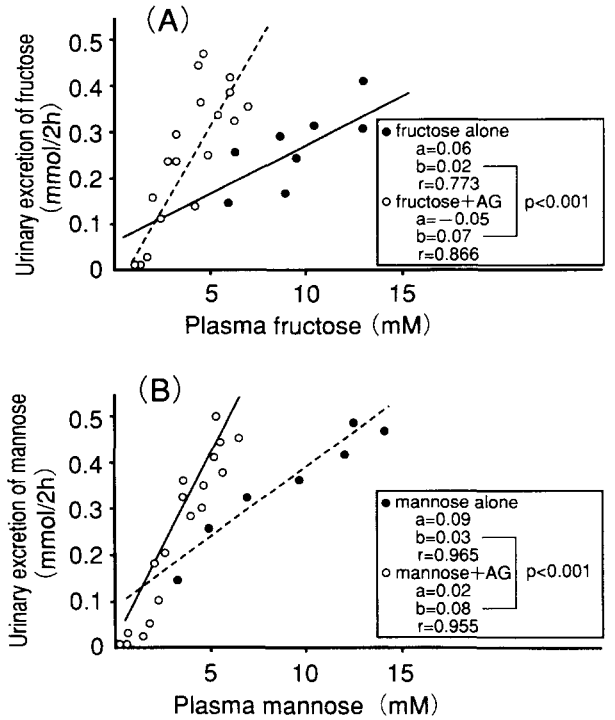

Fig. 5. Effect of AG on urinary excretion of fructose (A) and mannose (B). AG was infused at a concentration equal to or slightly higher than that of fructose or mannose (molar ratio: 1.0–5.0). Linear regression analysis revealed a positive correlation between the urinary excretion of fructose (mannose) and the plasma concentration of fructose (mannose), with or without an AG load. When the slopes of the linear regressions of these groups (b in the equation, $y = a + bx$) were compared, the value for b in the AG-loaded group significantly exceeded that of the groups administered fructose or mannose alone. This difference suggests that the change in slope was due to an acceleration of the excretion of fructose or mannose in the presence of a high plasma concentration of AG, as shown by the acceleration of AG excretion in the presence of glucose [4].

3.3. Overload of AG

We were unable to use large amounts of scarce AG. At plasma levels of AG that were equal to or slightly higher than those of glucose, neither the plasma nor the urinary concentration of glucose was influenced by the concentration of AG (data not shown). Under these conditions, renal retention (reabsorption) of AG exceeded 70% (Table 1).

4. Discussion

The structure of AG is similar to that of glucose. AG is excreted in the urine, and its serum concentration is decreased in patients with diabetes mellitus [21–24]. Its reabsorption by the renal tubule is competitively inhibited by glucose [2,4,25,26]. Whether the reabsorption of AG is mediated by a specific transporter, and whether sugars other than glucose interfere with its resorption is not known. We had observed a specific inhibition of AG reabsorption without glucosuria in patients receiving long-term hyperalimentation, suggesting the existence of a carrier system selective for AG [17]. The present study showed that the inhibition of AG reabsorption by both fructose and mannose was as strong as that produced by glucose. Because we observed no glucosuria during fructose or mannose loading, and because the amount of AG excreted in the urine almost equalled its calculated loss from the blood and body, as shown in a glucose-load balance study [2,4], a metabolic change of the loaded sugar to glucose and/or AG is not likely to be implicated. An increase in AG excretion in the urine was also observed in the xylose-loaded group, but this could be explained by the presence of glucosuria [2] related to the hyperglycemia induced by xylose. Under the present conditions, the amounts of both AG and glucose excreted in the urine after a single glucose load were compatible with those cited in previous reports [2,8] and the clinical evidence [4,7].

In addition to our preliminary experiments [27], competitive inhibition by mannose of AG reabsorption was also reported by Pitkänen et al. [28]. Their single intramuscular injection of mannose in rats resulted in the urinary excretion of AG; the urinary AG/mannose ratio was similar to our findings. In contrast, their long-term experiment, feeding a diet high in monosaccharide (one kind) to rats, showed different results [29]. Feeding rats galactose induced a drastic decrease in plasma AG, whereas fructose caused no change and xylose induced only a slight decrease. They failed to confirm these results in the intramuscular injection study cited above [28]. They acknowledged that the differences might arise from metabolic changes of the loaded sugar or from the hyperglycemia induced by the prolonged oral administration of unphysiologic amounts of a specific monosaccharide [28]. Furthermore, the plasma level of the loaded sugar that could be attained by intramuscular injection was so low that any specificity or affinity of the sugar for a transporter(s) could not be revealed.

The present study is the first to describe the effect on other sugars of plasma AG concentrations that were approx. 100 times the physiologic level. That this level of plasma AG did not increase the urinary excretion of glucose enabled us to exclude the possibility that reabsorption of other saccharides was interfered with indirectly by the increased glucosuria induced by the AG infusion. We also studied the affinity of an AG transporter for fructose or mannose at various plasma concentrations of these sugars produced by infusing a mixed-sugar load. Reabsorption of AG was little influenced by the presence of larger amounts of fructose or mannose, whereas the reabsorption of fructose or mannose was markedly inhibited by the presence of AG. An interaction between fructose and mannose was also observed. These findings suggest that the AG/fructose/mannose common transporter possesses a relatively high affinity for AG among these sugars. The physiological plasma concentration of AG was also rela-

tively high among these sugars. In the present experiments in rats, the basal plasma concentration of AG, fructose, and mannose was 55.7 ± 1.3, 9.8 ± 1.4, and 11.3 ± 1.4 μM, respectively. The existence of a selective carrier system for AG had been anticipated.

Glucose transport across epithelial cells of the kidney tubule is mediated by two Na^+-coupled glucose transporters termed SGLT [30], and by basolateral facilitated glucose transporters (GLUT) [31]. GLUT2 and GLUT5 each show a high capacity for fructose transport [32,33]. GLUT5 is now regarded to be the transporter exclusively for fructose [33]. The capacity of these transporters for mannose has not yet been evaluated. A mannose transporter [34] is suggested to exist in the mammalian renal tubule. The uptake system is moderately inhibited by glucose and phlorizin, and is strongly inhibited by fructose [35]. Pitkänen reported [28] that the resorption of mannose and AG is inhibited by phlorizin and 1,5-anhydro-mannitol. The glucose transporter of the rat [36], rabbit [37], and flounder [38] has shown little affinity for AG in vitro. Our observations are compatible with these results. Thus, it is strongly suggested that the reabsorption of AG is linked to that of mannose and fructose, which is distinct from the main system for glucose reabsorption. It had been assumed that AG is little taken up by cells, and that it is metabolically inert. However, an AG-specific transporter with a high K_m was recently found in rat hepatoma cells [9], human polymorphonuclear leukocytes [10], and the human erythroleukemia cell line K-562 [11]. Neither fructose nor mannose affected the facilitated AG transport system in K-562 cells. AG, a major polyol in the human body, thus shows unique biochemical and physiologic characteristics. Further investigation is needed to elucidate its pathophysiologic role.

References

[1] Kametani, S., Hasimoto, Y., Yamanouchi, T., Akanuma, Y. and Akanuma, H.(1987) J. Biochem. 102, 1599–1607.
[2] Yamanouchi, T., Akaoka, I., Akanuma, Y., Akanuma, H. and Miyashita, H. (1990) Am. J. Physiol. 258, E423–E427.
[3] Yamanouchi, T., Akanuma, H., Asano, T., Konishi, C., Akaoka, I. and Akanuma, Y. (1987) Diabetes 36, 709–715.
[4] Yamanouchi, T., Minoda, S., Yabuuchi, M., Akanuma, Y., Akanumal H., Miyashita, H. and Akaoka, I. (1989) Diabetes 38, 723–729.
[5] Yamanouchi, T., Akanuma, Y., Toyota, T., Kuzuya, T., Kawai, T., Kawazu, S., Yoshioka, S., Kanazawa, Y., Ohta, M., Baba, S. and Kosaka, K. (1991) Diabetes 40, 52–57.
[6] Yamanouchi, T., Tachibana, Y., Akanuma, H., Minoda, S., Shinohara, T., Moromizato, H., Miyashita, H. and Akaoka, I. (1992) Am. J. Physiol. 236, E268–273.
[7] Akanuma, Y., Morita, M., Fukuzawa, F., Yamanouchi, T. and Akanuma, H. (1988) Diabetologia 321, 831–835.
[8] Pitkänen, E. and Pitkänen, O. (1984) Experientia 40, 463–465.
[9] Suzuki, M., Akanuma, H. and Akanuma, Y. (1988) J. Biochem. 104, 956–959.
[10] Okuno, Y., Nishizawa, Y., Kawagishi, T., Sekiya, K., Shoji, T. and Morii, H. (1992) J. Biochem. 111, 99–102.
[11] Yamanouchi, T., Tachibana, Y., Sekino, N., Akanuma, H., Akaoka, I. and Miyashita, H. (1994) J. Biol. Chem. 296, 9664–9668.
[12] Crane, R.K. (1960) Physiol. Rev. 40, 789–825.
[13] Crane, R.K. (1962) Fed. Proc. 21, 891–895.
[14] Kleinzeller, A. (1970) Biochim. Biophys. Acta 211, 264–276.
[15] Silverman, M. and Black, J. (1975) Biochim. Biophys. Acta 394, 10–30.
[16] Hoshi, T. and Saito, Y. (1977) Contrib. Nephrol. 6, 52–60.
[17] Yamanouchi, T. Minoda, S., Ogata, N., Tachibana, Y., Sekino, N., Miyashita, H. and Akaoka, I. (1995) Clin. Sci. 88, 203–210.
[18] Okuda, J. and Miwa, I. (1971) Anal. Chem. 43, 312–315.
[19] Suzuki, M., Mizuno, H., Akanuma, Y. and Akanuma, H. (1994) J. Biochem. 115, 87–92.
[20] Yabuuchi, M., Masuda, M., Katoh, K., Nakamura, T. and Akanuma, H. (1989) Clin. Chem. 35, 2039–2043.
[21] Servo, C. and Pitkänen, E. (1975) Diabetologia 11, 575–580.
[22] Akanuma, Y., Ogawa, K., Yamanouchi, T., Mashiko, S., Oka, Y., Kosaka, K. and Akanuma, H. (1981) Diabetes 30, Suppl. 1,124A.
[23] Yoshioka, S., Saitoh, S., Negishi, C., Fujisawa, T., Fujimori, A., Takatani, O., Imura, M. and Funabashi, M. (1983) Clin. Chem. 29, 1396–1398.
[24] Yamanouchi, T., Akanuma, H., Nakamura, T., Akaoka, I. and Akanuma, Y. (1988) Diabetologia 31, 41–45.
[25] Yamanouchi, T., Akanuma, H., Takaku, F. and Akanuma, Y. (1986) Diabetes 35, 204–209.
[26] Yamanouchi, T, Moromizato, H., Shinohara, T., Minoda, S., Miyashita, H. and Akaoka, I. (1992) Metabolism 41, 862–867.
[27] Shinohara, T., Yamanouchi, T., Minoda, S., Irie, K., Moromizato, H., Tagaya, T., Yoshimura, R., Fujimori, S., Miyashita, H. and Akaoka, I. (1992) J. Jpn. Diab. Soc. 35, Suppl 1. 216 (in Japanese).
[28] Pitkänen, E. and Pitkänen, O.M. (1992) Pflugers Arch. 420, 367–375.
[29] Pitkänen, E. and Pitkänen, O.M. (1990) Experientia 46, 85–87.
[30] Kanai, Y., Wen-Sen, L., You, G., Brown, D. and Hediger, M.A. (1994) J. Clin. Invest. 93, 397–404.
[31] Kayano, T., Burant, C.F., Fukumoto, H., Gould, G.W., Fan, Y-S., Eddy, R.L., Byers, M.G., Shows, T.B., Seino, S. and Bell, G.I. (1990) J. Biol. Chem. 265, 13276–13282.
[32] Okuno, Y. and Gliemann, J. (1986) Biochim. Biophys. Acta. 862, 329–334.
[33] Inukai, K., Katagiri, H., Takata, K., Asano, T., Anai, M., Ishihara, H., Nakazaki, M., Kikuchi, M., Yazaki, Y. and Oka, Y. (1995) Endocrinology 136, 4850–4857.
[34] Silverman, M. (1981) Can. J. Physiol. Pharmacol. 59, 209–224.
[35] Mendelssohn, D.C. and Silverman, M. (1989) Am. J. Physiol. 257, F1100–F1107.
[36] Ullrich, K.J., Frömter, E., Hinton, B.T., Rumrich, G. and Kleinzeller, A. (1976) Curr. Probl. Clin. Biochem. 6, 256–261.
[37] Kleinzeller, A., McAvoy, E. and McKibbin, R.D. (1980) Biochim. Biophys. Acta 600, 513–529.
[38] Kleinzeller, A., Dubyak, G.R., Griffin, P.M., McAvoy, E.M., Mullin, J.M. and Rittmaster, R. (1977) Am. J. Physiol. 232, F227–F234.