Plasma 1,5-anhydroglucitol in experimental galactosemia in the rat

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Summary. Feeding with a galactose-rich diet induced a substantial drop in blood plasma 1,5-anhydroglucitol concentration. The decline was proportional to the dose of galactose. The decline was less marked in xylose-fed rats.
Key words. 1,5-anhydroglucitol; rat plasma; galactose; feeding response; xylose; fructose.

1,5-anhydroglucitol (AG) is a six-carbon monosaccharide with a pyranoid ring structure, which has a carbon skeleton isomerism identical with glucose, sorbitol and myo-inositol. AG is a major polyol in blood serum and cerebrospinal fluid[1-4]. The concentration of AG in serum shows no diurnal variation and is little affected by diet or short fasting[5], nor is the compound excreted in urine[6]. Studies in rats[7] have revealed that AG has a relatively long half-life in serum, which suggests that the compound is not an energy metabolite.

AG became a focus of interest owing to the observation that the concentrations were very low in the cerebrospinal fluid and serum in human Type 1 diabetes mellitus[1-3,8-10]. The concentration in serum remains low throughout the course of the disease and is not brought to the normal level by intensive insulin treatment[11]. Similarly low serum AG concentrations also characterize experimental diabetes mellitus, induced in rats by streptozotocin treatment[5,12,13]. Although data on this relationship has accumulated, the significance of the low levels for diabetic metabolism has proved to be elusive.

Recent studies have drawn attention to the metabolic similarities that exist between diabetes mellitus and experimental galactosemia induced by prolonged galactose feeding. Such similarities include activation of the sorbitol pathway[14], a decreased myo-inositol content in peripheral nerve and lens tissue[15], signs of early nephropathy[16], increased retinal vascular permeability[17,18] and thickening of the retinal vascular basal membrane[19-21], and prevention of the vascular changes by aldose reductase inhibitor[18-20]. This prompted us to check whether diabetes-like changes in the AG concentration may occur in experimental galactosemia.

Materials and methods

Wistar rats weighing 180–220 g were separated into groups of six animals each. The control rats received pulverized commercial chow of the following composition; moisture 11%, proteins 20%, lipids 3.5%, carbohydrates 54.5%, organic fiber material 5%, minerals and vitamins 6%. Diets containing 30%, 20% or 10% galactose were prepared by mixing D-galactose with the commercial chow (wt/wt). The test animals were given free access to the diet and water. The 30% and 20% diets elicited galactosuria which reached a maximum of 12% as tested with Clinitest reagent tablets (Ames, England). The 10% diet caused galactosuria up to 4.5%. The rats receiving the 30% diet had marked diuresis, which was obviously osmotic in origin. The level of galactose in random plasma samples was determined by means of gas liquid chromatography. The plasma galactose levels were 8.8–17.1 mmol/l (30% diet, n = 5), 1.4–4.8 mmol/l (20% diet, n = 3) and 1.4–2.3 mmol/l (10% diet, n = 3). The plasma galactitol levels were 0.6–1.6 mmol/l, 0.1–0.4 mmol/l and 0.1–0.2 mmol/l in the respective diet groups. No galactose or galactitol was detected in the samples of plasma collected from rats on commercial chow. Diets containing 30% D-glucose, D-xylose or D-fructose were prepared similarly.

Blood was collected through a small cut in the tail tip into heparinized glass capillaries (Vitrex capillaries, Modulohm, Denmark). The blood samples were quickly transferred into small centrifuge tubes and placed on ice. Plasma was collected after centrifugation (Greiner ZF 1 microcentrifuge, Greiner Electronic SA, Switzerland) and stored in a deep freeze (– 20 °C) until analyzed. The blood samples were collected in the morning whenever this was appropriate for the test design. Metabolic cages were used for the collection of the urine samples. Voided urine portions were harvested several times a day during the experiments to avoid fecal contamination. The urine samples were centrifuged without delay and stored at – 20 °C. Plasma and urinary AG as well as serum galactose and galactitol were determined by means of gas-liquid chromatography after conversion of the monosaccharides into peracetylated derivatives. Details of the method have been described previously[7,8]. A fused silica capillary column with CP-Sil-88 as the liquid phase (Chrompack, the Netherlands) was used in a Perkin-Elmer Sigma 3B chromatograph.

Data are reported as means ± SEM. Statistical differences were calculated using Student's t-test. $p > 0.01$ was used to determine statistical significance.

Results

The galactose-enriched diet caused a marked fall in the plasma AG concentration (fig. 1). The decline was gradual, taking place during the first week of the diet, after which the AG concentration stabilized at the new lower

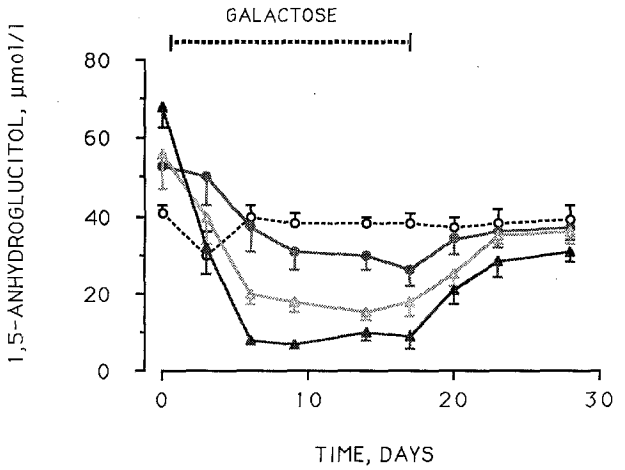

Figure 1. The response of plasma 1,5-anhydroglucitol to a galactose-rich diet. Three groups of rats were submitted for 17 days to 10%, 20% or 30% D-galactose diet. Serum AG was measured at intervals of 3 days. The results are presented as mean ± SEM (vertical bars). Open circles = rats on usual chow (n = 6); closed circles = 10% galactose diet (n = 6); light triangles = 20% galactose diet (n = 6); closed triangles = 30% galactose diet (n = 6).

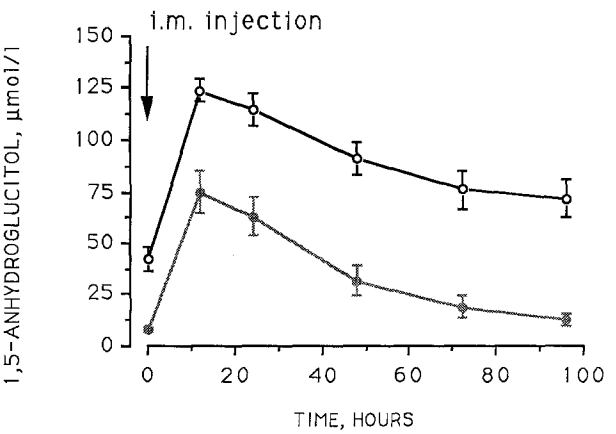

Figure 2. Plasma concentration after intramuscular administration (arrow) of 3 mg 1,5-anhydroglucitol to rats on normal chow (open circles, n = 6) and to rats on 30% galactose diet (closed circles, n = 6). Each diet was given for 14 days prior to the AG injection and the animals were on the respective diet throughout the study period. Plasma AG was monitored for 96 h at intervals of 12–24 h.

level. The decline was incremental, depending on whether the 10%, 20% or 30% diet was given. The decreases in AG were 51.4 ± 2.8% (p = 0.007, n = 6), 70.2 ± 2.8% (p = 0.003, n = 6) and 86.4 ± 2.5% (p = 0.0001, n = 6) of the pre-diet concentration, respectively (fig. 1). The decline in the control group was 3.9 ± 7.0% (not significant, n = 6). The galactose-enriched diets were given for 17 days after which the diets were replaced by the usual chow. This was accompanied by a slow increase in AG (fig. 1).

Six rats that had received the 30% galactose diet for two weeks and six rats fed the usual chow received 3 mg AG intramuscularly (fig. 2). The administration produced a rapid increase in the plasma AG concentration followed by a gradual decline. The slopes gave T1/2-values of 31.8 ± 2 h for the galactose-treated rats and 120 ± 27 h for the controls (p for the difference = 0.0089). Of the dose administered, 25 ± 4% was recovered in the urine of galactose-fed rats during the first 24 h whereas the excretion was lower in the rats fed the usual chow (6 ± 1%; p = 0.001).

In order to see whether the plasma AG concentration in galactose-fed animals can be maintained at normal levels by oral AG substitution, three rats were given a 30% galactose diet for a week, followed by two consecutive weeks during which the rats were given the 30% galactose diet enriched with 50 mg AG/kg diet (one week) and with 25 mg/kg (one week). The estimated daily food intake per rat was 30 g, and the likely approximate daily AG doses per animal were 1.6 and 0.8 mg, respectively. As summarized in table 1, the pretreatment AG concentration was attained with both of the two dosages. A small amount of AG (0–0.4 mg in 24 h) was recovered in the urine during the period of the larger AG dose.

Table 1. Plasma 1,5-anhydroglucitol concentration during oral substitution in galactose-fed rats

Diet	1,5-Anhydroglucitol (µmol/l)		
Rat	1	2	3
Usual chow	34	25	35
30% galactose	11	3	8
30% galactose with 50 mg/kg 1,5-anhydroglucitol	61	29	49
30% galactose with 25 mg/kg 1,5-anhydroglucitol	38	26	40

Each diet modification was given consecutively in periods of one week.

Table 2. Effect of diets enriched with glucose, fructose or xylose on the plasma 1,5-anhydroglucitol concentration

	1,5-anhydroglucitol (µmol/l)		
Diet	Before treatment	Diet for two weeks	p
Usual chow	38.2 ± 1.8	37.6 ± 3.2	NS
30% glucose	65.9 ± 11.4	72.1 ± 9.4	NS
30% fructose	42.8 ± 6.4	35.0 ± 5.4	NS
30% xylose	36.6 ± 2.6	26.8 ± 1.7	0.013

Each reading consists of the mean taken from 6 animals (± SEM).

No peak attributable to 1,5-anhydrogalactitol, which is the 1,5-anhydroisomer of D-galactose, was detected in any of the chromatograms prepared from the plasma of galactose-fed rats.

To establish whether the fall in AG was a specific characteristic of galactose, diets enriched with 30% glucose, fructose or xylose were given to three groups of rats, with six rats in each. Plasma AG was determined prior to the institution of the diet and at the end of a diet period of two weeks. The findings are summarized in table 2. There was a fall in plasma AG in rats on the xylose diet (27.8% decrease; p = 0.013). The decline was modest in comparison with the drop elicited by any of the galactose diets used (fig. 1).

Discussion

The salient observation of the present study was the marked decline in the plasma AG concentration resulting from galactose feeding. The low values induced by 30% galactose are similar to the low values characteristic of both human[8,10] and experimental[4,5,13] diabetes mellitus. The drop depended on the amount of galactose and might have been even more pronounced if 35–50% galactose diets had been used. Such diets have also been given to elicit microangiopathy in experimental galactosemia[17–19].

A sensitive measurement of urinary AG in the present study was hampered by the considerable amounts of galactose and galactitol in the samples. This jeopardized the determination of baseline AG excretion, which implies that a small excretion of AG during galactose feeding cannot be excluded. However, an accelerated elimination rate was revealed by the short T1/2 time of exogenous AG in galactose-fed rats when AG was administered by intramuscular injection (fig. 2), associated with high urinary excretion of AG. This indicates that the urinary excretion was a major route of AG elimination in galactose-fed rats, contributing to the decline in plasma AG. Earlier studies[7] on the time course of AG excretion in untreated rats have indicated that AG is efficiently reabsorbed by rat kidney and that the reabsorption mechanism is saturable, making it possible to establish a threshold value for the urinary excretion. The higher urinary excretion and the lower plasma concentration of exogenous AG in the galactose-treated rats throughout the test period in the present study (fig. 2) seem to imply that the renal threshold value was markedly lowered, or that galactose and AG compete for a common transport mechanism in the kidney tubuli. Further studies are necessary to clarify this point.

The data on the T1/2 times and the fact that the plasma AG concentration was maintained at the pretreatment level by oral substitution with small AG dosages (table 1) reveal that the galactose-fed rats had retained a considerable capacity to reclaim urinary AG. This keeps open the possibility that the synthesis of AG may have been reduced by galactose, thereby contributing to the drop in plasma AG. The main pathway of galactose metabolism involves the conversion of galactose to glucose through a series of reactions involving galactokinase and sugar nucleotides. Accessory pathways are activated in the presence of a large load of galactose. An oxidative pathway related to the formation of galactonic acid[22] is well established, although the enzymatic machinery of the pathway is still under dispute. The polyol (sorbitol) pathway, in which aldose reductase is the key enzyme, constitutes a further alternate pathway activated by galactose feeding. Galactose is reduced to galactitol through this metabolic route. The pathway is very active both in human[23] and experimental[14,18–20] galactosemia, since large amounts of galactitol accumulate in serum, urine and several tissues in both conditions. A stimulated sorbitol pathway is also a landmark feature in experimental diabetes[24], as glucose is transformed to sorbitol and fructose through the pathway during hyperglycemia. The coexistent changes in the AG concentration and in the sorbitol pathway in both of the disease states, as well as the fact that the sorbitol pathway involves compounds stereochemically closely related to AG, warrants further studies for detecting a possible linkage between AG synthesis and the sorbitol pathway.

Studies with diets enriched with glucose, fructose and xylose revealed that the xylose diet induced a fall in plasma AG concentration with only borderline significance ($p = 0.013$). However, it may be inferred from the results that the drastic decline in AG was specific to galactose feeding. There was no reduction in AG when the glucose-rich diet was used, indicating that the fall of AG could not be attributed to a change in metabolism in order to consume a more carbohydrate-rich fuel.

Galactose feeding offers an opportunity to carry out long-term metabolic studies in the presence of very low AG concentrations in vivo, and to link them to intervention studies with exogenous AG. This is a way in which the elucidation of the enigmatic metabolic function of AG can be approached.

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