Renal tubular reabsorption of 1,5-anhydro-D-glucitol and D-mannose in vivo in the rat

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Abstract. 1,5-Anhydro-D-glucitol (AG) is efficiently reabsorbed in renal tubuli by a mechanism that is saturated at high AG concentrations. To gain insight into the stereospecific requirements of the mechanism, we employed an in vivo loading test technique in which rats were injected with anhydrosugars and aldohexoses in doses that led to excretion of the sugar injected, thus saturating tubular reabsorption. Administration of AG elicited an increase in the excretion of D-mannose (P<0.0005), while D-mannose caused AG to appear in urine. Administration of 1,5-anhydro-D-mannitol led to increased excretion of D-mannose (P<0.0005) and the appearance of AG in urine. The effects of 1,5-anhydro-D-mannitol on the excretion of D-mannose and AG, and the effect of D-mannose on AG were dependent on the dose. Myoinositol, mannitol and C-3- C-6 epimers of AG did not interfere with the reabsorption. The mechanism was highly phlorizin-sensitive. Repeated administration of 1,5-anhydro-D-mannitol rapidly depleted the rat organism from mobilizable AG. The AG space calculated (53% of body weight) suggested the presence of considerable cellular stores of AG. D-Mannose and AG are regular components of the plasma monosaccharide profile. The data suggest that the two sugars are reabsorbed in renal tubuli by a common mechanism, which is distinct from the main D-glucose reabsorption system. The mechanism accommodates both an axial and an equatorial hydroxyl group at C-2, but a hydroxyl group at C-1 is not required.

Key words: D-Mannose – 1,5 Anhydro-d-glucitol – Renal tubular reabsorption – In vivo – Loading test – Stereospecific requirements – Anhydrosugars – Rat

Introduction

1,5-Anhydro-D-glucitol (AG), a major polyol in human and rat blood plasma [25, 30], is freely filtered into the tubular fluid by kidney glomeruli. The AG concentration in urine is much lower than the concentration in plasma [19], indicating that AG is effectively reabsorbed by tubuli. The reabsorption mechanism is saturated at high plasma AG concentrations [19]. Efficient renal reclamation of AG is obviously vital in maintaining physiological AG concentrations in blood, considering the very slow rate of synthesis of AG in the rat organism [19].

The in vivo properties of the tubular carrier mechanism involved in AG reabsorption are still uncharted. The anhydro ring of the AG molecule seems to be a unique feature distinguishing AG from all other sugars in the rat and human organism. This sparked our interest to study the transport system in order to define its stereospecific characteristics. The present study investigated the effect on urinary AG excretion of acute administration of anhydropolysols and hexoses carrying isomeric structures close to AG. The dose of each sugar injected was established in preliminary studies to produce overflow excretion of the sugar injected. Marked excretion of the injected sugar was taken to indicate that the tubular reabsorption mechanism by which the sugar may be reabsorbed was fully saturated. The study revealed that reabsorption of AG is linked to reabsorption of D-mannose, another hexose consistently present in plasma and reclaimed by the kidney.

Materials and methods

Preparation of sugars. 1,5-Anhydro-D-glucitol from acetobromo-D-glucose, 1,5-anhydro-D-mannitol from α-D-mannopyranose pentaacetate, 1,5-anhydro-D-galactitol from β-D-galactopyranose pentaacetate and 1,5-anhydro-L-glucitol from β-L-glucopyranose pentaacetate were prepared using lithium aluminum hydride reduction of acetylated glycopyranosyl bromides [17], β-D-Allopyranose, 6-deoxy-D-glucose and D-xylene were acetylated by treatment with acetic anhydride and pyridine. The peracetylated derivatives were converted to the bromide form and reduced [17] to produce 1,5-anhydro-D-allitol, 1,5-anhydro-6-deoxy-D-
glucitol and 1,5-anhydroxylitol respectively. The D-xylose was from E. Merck, Darmstadt, FRG, the other parent compounds were from Sigma Chemical Company, St. Louis, USA. The hydrobromic acid in acetic acid was purchased from Fluka AG, Buchs, Switzerland. 1,4-Anhydro-D-glucitol was manufactured from D-sorbitol by acid-catalysed dehydration [6]. Each anhydropolyol product was recrystallized one to three times from hot ethanol, except for 1,5-anhydro-6-deoxy-D-glucitol, which was crystallized from hot n-butanol, to obtain preparations that were pure when studied by gas-liquid chromatography and thin-layer chromatography [18]. The mass fragmentograms of the peracetylated products are given in Fig. 1.

Recrystallization of phlorizin. Phlorizin (Sigma Chemical Company, St. Louis, USA) was dissolved in ethanol. Hot water was added to the ethanol solution, and recrystallization was allowed to proceed in the refrigerator overnight [13].

Experimental technique. Six male Wistar rats weighing 210—250 g were used for each experiment. The rats were given no food for 10 h before each experiment. Blood samples were taken immediately before injection, and during the tests at the intervals shown separately in Results. The rats were placed in metabolic cages for urine collection, which was conducted for 4, 7 or 12 h. The techniques of taking tail-tip blood samples and collecting urine were described in detail in a previous report [20]. The test sugars were injected intramuscularly in the form of 5% solutions in water. Phlorizin was injected intramuscularly as a fresh 2% solution in 2% sodium bicarbonate in water [12]. Furosemide (10 mg/ml) was injected intramuscularly.