Effect of $\alpha$-glucosidehydrolase inhibition on intestinal absorption of sucrose, water, and sodium in man

W. F. Caspary and H. Kalisch

From the Division of Gastroenterology and Metabolism, Department of Medicine, University of Göttingen, Germany

SUMMARY The effect of a new complex oligosaccharide exhibiting potent inhibitory action on $\alpha$-glucoside hydrolases on intestinal absorption of sucrose in man was tested by constant in vivo perfusion of the jejunum. At concentrations of 4.65 or $15.5 \times 10^{-4} \text{M}$ the $\alpha$-glucosidehydrolase inhibitor ($\alpha$-GHI) markedly inhibited absorption of glucose from sucrose and absorption of sodium and water. Oral administration of the $\alpha$-GHI resulted as well in depression of solute, sodium, and water absorption. This new compound can serve as an interesting tool to induce carbohydrate malabsorption by inhibition of final digestion and may possibly be of beneficial therapeutic effect in dietary control of diabetes or obesity.

Terminal digestion of carbohydrates by $\alpha$-glucosidehydrolases and absorption of hydrolytic products are closely linked processes at the brush border membrane of mucosal epithelial cells (Gray, 1975; Crane, 1975; Caspary, 1977a). A recently described complex oligosaccharide of microbial origin (molecular weight 645) was found to possess potent inhibitory action on $\alpha$-glucosidehydrolases from pig intestine (Schmidt et al., 1977). The $K_i$ of this potent $\alpha$-glucosidehydrolase inhibitor ($\alpha$-GHI) was found to be $1.3 \times 10^{-4} \text{M}$ versus sucrose from human intestine—that is, the inhibitor had a more than 10,000-fold higher affinity to the enzyme sucrase than its natural substrate sucrose (Caspary et al., 1978). The affinity ($K_i$) of this oligosaccharide to intestinal sucrase is therefore in the order of magnitude of phlorizin-interaction with the glucose carrier in the brush border membrane (Alvarado and Crane, 1962).

This study was designed to test whether the $\alpha$-GHI will inhibit absorption of glucose derived from sucrose in healthy human volunteers under conditions of in vivo intestinal perfusion.

Methods

GENERAL
Intestinal perfusion studies were performed in 10 healthy volunteers (mean age: $25 \pm 7$ years) who were informed about the details of the study and gave written consent. Approval for the study was given by a local University Ethical Committee.

Two different designs of studies were used: (1) perfusion of a 30 cm jejunal segment with a double lumen tube incorporating a proximal occluding balloon to test the direct effect of the $\alpha$-GHI in the perfusate on intestinal absorption of glucose from sucrose, water, and sodium; (2) perfusion of a 30 cm jejunal segment with a triple lumen tube containing a 10 cm mixing segment in order to follow the effect of oral administration of the $\alpha$-GHI on intestinal absorption.

STUDY I (Fig. 1)
Perfusion of the proximal 30 cm of jejunum was carried out with a double lumen tube incorporating a proximal occluding balloon. Positioning of the tube was accomplished by fluoroscopy until the proximal occluding balloon was just beyond the ligament of Treitz. The perfusate consisted of: sodium chloride 130 mmol/l, sucrose 50 mmol/l, polyethylene glycol (PEG 4000) 4 g/l, $^{14}$C-polyethylene glycol (New England Nuclear, Dreieichenhain, Germany, spec. act.: 1 mCi/g) 1000 dpm/ml. Solutions were perfused at a constant rate of 10 ml/min. After a 30 minute equilibration period three 10 minute collections were made from the distal end of the test segment. All
samples were collected on ice, centrifuged, and stored at −20°C. Thereafter perfusion was continued for an additional 60 minutes with the same perfusate containing in addition 3 μg/ml (±4·65 × 10⁻⁴M) of the α-glucosidehydrolase inhibitor (α-GHI) kindly provided by Professor L. P. Berchtold, Dr. L. Hillebrand and Dr. T. W. Puls (Bayer Pharmaceutical Corporation, Wuppertal, Germany). Thirty minutes were allowed for equilibration and three 10 minute collections were sampled for analysis. A third perfusion period over an additional 60 minutes was performed with the addition of 10 μg/ml (±15·5 × 10⁻⁴M) of the α-GHI. Equilibration time and sampling was as before.

STUDY II (Fig. 1)
In a second series of experiments performed in the same subjects the proximal jejunum was intubated with a triple lumen tube by fluoroscopic control. The infusion site of the tube was at the area of the ligament of Treitz, with aspirating sites 10 and 40 cm distal to the infusion opening in the jejunum. The perfusion solution was the same as in study I (perfusion rate: 10 ml/min). After a 30 minute equilibration period three 10 minute collections were obtained from the proximal and distal aspiration sites. After 60 minutes 300 mg of the α-GHI dissolved in 75 ml of water were given by mouth and the perfusion was continued by obtaining 10 minute collections for another 110 minutes. Aspirated samples were processed after deproteinisation.

ANALYSIS
Analysis for D-glucose and sucrose was performed by measuring D-glucose (hexokinase method) before and after complete hydrolysis of sucrose by fructosidase (sucrose/glucose test kit No. 15824; Boehringer Mannheim). ¹⁴C-PEG was assayed by a Packard liquid scintillation system with automatic quench correction. Sodium was determined by flame photometry (KLINA flame photometer, Beckman Instruments, Munich). Net absorption rates of glucose from sucrose, water, and sodium were calculated using standard formulas (Whalen et al., 1966). Statistical analysis of the data was performed by Student’s t test.

Results
The presence of 3 or 10 μg/ml (±4·65 or 15·5 × 10⁻⁴M) of the α-GHI resulted in a marked decrease of net jejunal absorption of glucose from sucrose, sodium, and water (Fig. 2). Mean glucose absorption decreased from 5139 ± 161 (SEM) μmol/10 min per segment to 3810 ± 147 μmol/10 min per segment (p < 0·001) in the presence of 3 μg/ml of the α-GHI and to 2509 ± 188 μmol/10 min per segment (p < 0·001) at 10 μg/ml of the α-GHI. Water and
sodium absorption were also markedly reduced in the presence of the α-GHI in the perfusate. At the concentration of 10 μg/ml α-GHI a net secretion of water was observed.

Absorption of glucose from sucrose (50 mmol) measured by the triple lumen tube incorporating a mixing segment was lower compared with absorption of glucose measured with the double lumen technique (Figs. 2 and 3).

Oral administration of 300 mg of the α-GHI dissolved in 75 ml of water resulted in decreased absorption of glucose from sucrose during the third 10 minute sampling period after oral α-GHI administration (Fig. 3). The inhibitory effect of α-GHI administration persisted up to 170 minutes (110 minutes after administration of the α-GHI) (Fig. 3). A similar pattern of inhibition was observed for water and sodium absorption (Figs. 4 and 5). At 100 minutes

---

**Fig. 3** Jejunal absorption of glucose from sucrose before and after oral administration of 300 mg of an α-glucosidasehydrolase inhibitor. Perfusion of a 30 cm jejunal segment (study II) was performed with a triple lumen tube incorporating a 10 cm mixing segment. Orinate: absorption of glucose expressed as μmol/30 cm segment x 10 minutes. Results are means±SEM (n=10).

---

**Fig. 4** Effect of oral administration of the α-GHI on jejunal absorption of water from a sodium chloride-sucrose perfusate. Experimental design as in Fig. 3. Ordinate: water absorption expressed as ml/30 cm segment x 10 minutes. Results are means±SEM (n=10).
Effect of α-glucosidehydrolase inhibition on intestinal absorption of sucrose, water, and sodium in man

Discussion

Intestinal absorption of hexoses may be depressed in humans by biguanides (Arvanitakis et al., 1973; Caspary, 1977b), prenylamine (Caspary and Creutzfeldt, 1972; Gottesburen et al., 1974), and aspirin (Arvanitakis et al., 1977). Inhibition of sodium-dependent active hexose transport by depriving mucosal cells of energy seems to be the underlying mechanism responsible for the inhibitory action of biguanides and aspirin on intestinal glucose absorption (Arvanitakis et al., 1977; Caspary, 1977b).

Another approach to inhibit glucose absorption (from sucrose) has been reported with the use of TRIS which is a competitive inhibitor of sucrase (Puls and Keup, 1975). A recently developed new α-glucosidehydrolase inhibitor possessed potent inhibitory action on α-glucosidase activities from pig small intestine (Schmidt et al., 1977), rat and human small intestine (Caspary, 1978a; Caspary et al., 1978). This potent inhibitor of human γ-amylase, sucrase, and maltase has an affinity to sucrase which lies in the same range phlorizin interacts with the glucose carrier binding site (Alvarado and Crane, 1962). It should therefore be able to decrease intestinal absorption of glucose from disaccharides, especially from sucrose. Oral loading with sucrose or maltose in humans resulted in a decrease of postprandial hyperglycaemia when the α-glucosidehydrolase inhibitor was given, thus suggesting inhibition or delay of absorption (Puls et al., 1977; Caspary, 1978b).

Under perfusion conditions with the double lumen tube including a proximal balloon steady state perfusion conditions were obtained after 30 minutes of perfusion. The presence of the α-GHI in the perfusate (study 1) resulted in a 25-85% decrease of glucose absorption from sucrose at an inhibitor concentration of 4·85 × 10⁻⁶M and in 51-16% decrease in the presence of 15·5 × 10⁻⁶M of the α-GHI. Water and sodium absorption were decreased to an even greater extent (Fig. 2). Decreased absorption of disaccharides in lactase- or sucrase-isomaltase deficient subjects (Launiala, 1968; Kern and Struthers, 1966) or of D-glucose after pretreatment with phenformin (Arvanitakis et al., 1973) resulted in a considerably more extensive inhibition or even in secretion of net water and sodium transport than reduction of solute (glucose) absorption. Thus the unsplit disaccharides exerted an osmotic effect which may induce secretion of water depending on the amount of maldigested disaccharides (Launiala, 1968).

As in vitro studies with rat intestine (Caspary, 1978a) have shown that the α-GHI interferes only with α-glucosidehydrolases (γ-amylase, sucrase, maltase), but not with the intestinal active hexose transport system itself, it can be assumed that inhibition of glucose absorption from sucrose was due to inhibition of absorption of the hydrolytic products of sucrose. Pilot studies performed in three patients had shown that the presence of 15·5 × 10⁻⁶M α-GHI in the perfusate did not affect absorption of free glucose. As, in addition, concentration of free glucose measured in the aspirate was minimal in the presence and in the absence of the α-GHI, it can be concluded that inhibition of glucose absorption from sucrose was entirely due to inhibition of sucrose hydrolysis.
The aim of the second study (study II) was to test how long the inhibitor's action would persist after a single oral dose. Initially, it was intended to take small bowel biopsies at different time intervals after an oral dose of the inhibitor in order to measure inhibition of α-glucosidases in the biopsy tissue. The flushing buffer volume to obtain the biopsies by an hydraulic biopsy device and the dilutions during processing of the biopsy material for enzyme analysis would certainly have resulted in dissociation of the competitive inhibitor from the enzymes. We therefore used the constant perfusion technique with a non-absorbable marker (triple lumen technique). This technique requires steady state conditions—that is, a constant concentration of dilutional marker in the perfusate (Levitt and Bond, 1977). The steady state requirement was fulfilled during the control period when sucrose alone was perfused with the marker (PEG 4000). Levitt and Bond (1977) have clearly pointed out that the constant perfusion technique in the non-steady state may lead to errors. After administration of the α-GHI with 75 ml of water, steady state conditions were apparently no longer maintained because of the volume of water added and the concentration-dependent action of the inhibitor on sucrose hydrolysis. Nevertheless, we used the constant perfusion technique in the non-steady state because we could not think of any other approach to answer the question posed in study II. The magnitude of inhibition of glucose absorption from sucrose, of absorption of water and sodium was so high that possible errors imposed by the non-steady state seem to be negligible.

Inhibition of glucose absorption from sucrose was more pronounced after oral administration of 300 mg of the α-GHI than by direct and constant presence of 10 μg/ml of the α-GHI in study I. The actual concentration of the α-GHI during perfusion in study II is not known, but assuming dilution of the α-GHI by at least 1000 ml of water, gastric, pancreatic, and biliary secretions and perfusate it could be deduced that at least for some time of the perfusion period the concentration might have reached 300 μg/ml, a concentration 30-fold higher than under the conditions of study I.

Additional studies in the meantime have shown that administration of the α-GHI to humans not only induced a delay of glucose absorption from sucrose but resulted in maldigestion of sucrose measured by massive breath hydrogen production; this was observed in subjects ingesting 100 g of sucrose together with 200 mg of the α-GHI (Caspar, 1978b) in the same way as in patients with hyposucrasia ingesting sucrose (Metz et al., 1976). Six of the 10 volunteers undergoing study I and five out of 10 participating in study II developed diarrhoea and flatulence toward the end of the perfusion period.

The new α-GHI can serve as an interesting tool to mimic final maldigestion of α-glucosides without affecting monosaccharide absorption. It is possible that the inhibitor may prove to be of benefit in the dietary control of diabetes or obesity and it is currently being tested by several European groups. A dose response study will have to show whether a delay of glucose absorption from carbohydrates induced by the α-GHI without inducing malabsorption still has beneficial effects in reducing postprandial blood glucose.

References


Effect of α-glucosidehydrolase inhibition on intestinal absorption of sucrose, water, and sodium in man


