Effect of α -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 α -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 15.5×10^{-6} M the α -glucosidehydrolase inhibitor (α -GHI) markedly inhibited absorption of glucose from sucrose and absorption of sodium and water. Oral administration of the α -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 α -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 α -glucosidehydrolases from pig intestine (Schmidt *et al.*, 1977). The K_i of this potent α -glucosidehydrolase inhibitor (α -GHI) was found to be 1.3×10^{-6} M versus sucrase 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 α -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 ± 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 α -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 α -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

Received for publication 5 June 1979

Fig. 1 Synopsis of designs of study I and study II. For details see text (Methods).

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 $\mu\text{g/ml}$ ($=4.65 \times 10^{-6}\text{M}$) 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 $\mu\text{g/ml}$ ($=15.5 \times 10^{-6}\text{M}$) 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

Fig. 2 Effect of an α -glucosidehydrolase inhibitor (α -GHI) on intestinal absorption of glucose from sucrose, water and sodium. A 30 cm jejunal segment was perfused with 50 mmol sucrose (study I) in the absence (control: open bar) and in the presence of 3 (second bar) or 10 $\mu\text{g/ml}$ of an α -glucosidehydrolase inhibitor. Results are means $\pm$ SEM ($n=10$). ■ $p < 0.001$; ■■ $p < 0.005$.

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). ^{14}C -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 $\mu\text{g/ml}$ ($=4.65$ or $15.5 \times 10^{-6}\text{M}$) 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) $\mu\text{mol}/10$ min per segment to 3810 ± 147 $\mu\text{mol}/10$ min per segment ($P < 0.001$) in the presence of 3 $\mu\text{g/ml}$ of the α -GHI and to 2509 ± 188 $\mu\text{mol}/10$ min per segment ($P < 0.001$) at 10 $\mu\text{g/ml}$ of the α -GHI. Water and

Fig. 3 Jejunal absorption of glucose from sucrose before and after oral administration of 300 mg of an α -glucosidase inhibitor. Perfusion of a 30 cm jejunal segment (study II) was performed with a triple lumen tube incorporating a 10 cm mixing segment. Ordinate: absorption of glucose expressed as $\mu\text{mol}/30\text{ cm segment} \times 10\text{ minutes}$. Results are means $\pm$ SEM ($n=10$).

sodium absorption were also markedly reduced in the presence of the α -GHI in the perfusate. At the concentration of 10 $\mu\text{g}/\text{ml}$ 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. 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 $\text{ml}/30\text{ cm segment} \times 10\text{ minutes}$. Results are means $\pm$ SEM ($n=10$).

Fig. 5 Effect of oral administration of an α -GHI on intestinal absorption of sodium from a sodium chloride-sucrose perfusate. Experimental design as in Fig. 3. Ordinate: sodium absorption expressed as $\mu\text{mol}/30\text{ cm segment} \times 10\text{ minutes}$. Results are means $\pm$ SEM

a slight net secretion of water and sodium was found (40 minutes after α -GHI-administration).

Discussion

Intestinal absorption of hexoses may be depressed in humans by biguanides (Arvanitakis *et al.*, 1973; Caspary, 1977b), prenylamine (Caspary and Creutzfeldt, 1972; Gottesbüren *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 I) resulted in a 25.85% decrease of glucose absorption from sucrose at an inhibitor concentration of $4.85 \times 10^{-6}\text{ M}$ and in 51.16% decrease in the presence of $15.5 \times 10^{-6}\text{ M}$ 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 $15.5 \times 10^{-6}\text{ M}$ 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 μ g/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 μ g/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 malabsorption 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 (Caspary, 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

- Alvarado, F., and Crane, R. K. (1962). Phlorizin as a competitive inhibitor of the active transport of sugars by hamster small intestine. *Biochimica et Biophysica Acta*, **56**, 170-180.
- Arvanitakis, C., Chen, G. H., Folscroft, J., and Greenberger, N. J. (1977). Effect of aspirin on intestinal absorption of glucose, sodium, and water in man. *Gut*, **18**, 187-190.
- Arvanitakis, C., Lorenzsonn, V., and Olsen, W. A. (1973). Phenformin-induced alterations of small intestinal function and mitochondrial structure in man, *Journal of Laboratory and Clinical Medicine*, **82**, 196-200.
- Bond, J. H., and Levitt, M. D. (1977). Use of breath hydrogen (H_2) in the study of carbohydrate absorption. *American Journal of Digestive Diseases*, **22**, 379-382.
- Caspary, W. F. (1977a). Mechanism and specificity of intestinal sugar transport. In *Intestinal Permeation*, pp 74-83. Edited by M. Kramer and F. Lauterbach. Excerpta Medica: Amsterdam.
- Caspary W. F. (1977b). Biguanides and intestinal absorptive function. *Acta Hepato-Gastroenterologica*, **24**, 473-480.
- Caspary, W. F. (1978a). Potent inhibition of intestinal sucrase, γ -amylase, and maltase by a new α -glucosidase inhibitor (α -GHI). Meeting of the European Society of Clinical Investigation, Rotterdam, April 1978.
- Caspary, W. F. (1978b). Sucrose-malabsorption in man after ingestion of α -glucosidehydrolase inhibitor. *Lancet*, **1**, 1231-1233.
- Caspary, W. F., and Creutzfeldt, W. (1972). Hemmung der intestinalen Resorption von Zuckern und Aminosäuren durch Prenylamin (Segontin[®]). *Deutsche Medizinische Wochenschrift*, **97**, 394-396.
- Caspary, W. F., Graf, S., and Sido, B. (1978). Neue Möglichkeiten zur Hemmung von α -Glucosidasen in der Dünndarmschleimhaut. 84. *Tagung der Deutschen Gesellschaft für Innere Medizin*, Wiesbaden, 1978.
- Crane, R. K. (1975). Fifteen years of struggle with the brush border. In *Intestinal Absorption and Malabsorption*, pp 127-142. Edited by T. Z. Csaky. Raven Press: New York.
- Gray, G. M. (1975). Carbohydrate digestion and absorption. Role of the small intestine. *New England Journal of Medicine*, **292**, 1225-1230.

- Gottesbüren, H., Raida, H., and Riecken, E. O. (1974). Untersuchungen zum Einfluß von Prenylamin auf die Dünndarmresorption beim Menschen. *Deutsche Medizinische Wochenschrift*, **99**, 2104-2105.
- Kern, F., and Struthers, J. E. Jr. (1966). Intestinal lactase deficiency and lactose intolerance in adults. *Journal of the American Medical Association*, **195**, 927-930.
- Launiala, K. (1968). The mechanism of diarrhoea in congenital disaccharide malabsorption. *Acta Paediatrica Scandinavica*, **57**, 425-432.
- Levitt, M. D., and Bond, J. (1977). Use of the constant perfusion technique in the non steady state. *Gastroenterology*, **73**, 1450-1454.
- Metz, G., Jenkins, D. J. A., Newman, A., and Blendis, L. M. (1976). Breath hydrogen in hyposucrasia. *Lancet*, **1**, 119-120.
- Puls, W., and Keup, U. (1975). Inhibition of sucrase by TRIS in rats and man, demonstrated by oral loading tests with sucrose. *Metabolism*, **24**, 93-98.
- Puls, W., Keup, U., Krause, H. P., Thomas, G., and Hoffmeister, F. (1977). Glucosidase inhibition. A new approach to the treatment of diabetes, obesity, and hyperlipoproteinaemia. *Naturwissenschaften*, **64**, 536.
- Schmidt, D. D., Frommer, W., Junge, B., Müller, L., Wingender, W., Truscheit, E., and Schäfer, D. (1977). α -glucosidase inhibitors. New complex oligosaccharides of microbial origin. *Naturwissenschaften*, **64**, 535.
- Whalen, G. E., Harris, J. A., Geenen, J. E. and Soergel, K. H. (1966). Sodium and water absorption from the human small intestine. The accuracy of the perfusion method. *Gastroenterology*, **51**, 975-984.