Cholecystokinin Decreases Intestinal Hexose Absorption by a Parallel Reduction in SGLT1 Abundance in the Brush-Border Membrane

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The dual luminal and vascularly perfused small intestine was used to determine the mechanism by which cholecystokinin octapeptide (CCK-8) decreases the rate of glucose absorption. With CCK-8 in the vascular perfusate the rate of 3-O-methyl-D-glucose absorption decreased, whereas the rate of n-fructose absorption was unaffected. The substrate pool size within the tissue during steady-state transport, in the presence and absence of CCK-8, was estimated by compartmental analysis of the 3-O-methyl-D-glucose washout into the vascular bed. When CCK-8 was included in the vascular perfusate, the absorptive cell pool size decreased when compared with untreated tissue. Both the steady-state hexose absorption data and the washout studies indicated that the locus of action of CCK-8 was the SGLT1 transporter located in the brush-border membrane. The SGLT1 protein abundance in isolated brush-border membranes, as quantified by Western blotting, showed a decrease that paralleled the decrease in the steady-state transport rate induced by CCK-8. These results indicate that CCK-8 diminishes the rate of intestinal hexose absorption by decreasing SGLT1 protein abundance in the brush-border membrane of the rat jejunum and therefore provides evidence for acute enteric hormonal regulation of the rate of glucose absorption across the small intestine.

The systemic plasma glucose concentration is normally maintained within a fairly narrow range (4.5–6.5 mM) even during episodic eating. The regulation of this homeostatic process is provided by multiple systems in the body. One of the systems involved in delivering glucose to the systemic circulation through the absorption of carbohydrate-digested products is the small intestine. To this end, the rate at which hexoses are sorbed aldoses is subjected to rapid and specific enteric peptide regulation involving the enteric peptide-2 (5–7). However, mechanism(s) involved in the CCK-induced decrease of hexose absorption have not been identified (4). Possible mechanisms involved in altering hexose transport rates include changes in the electrochemical gradient for sodium (8), the affinity of the transporter for glucose (9), and the amount of functional transporter present in the membrane (10). Recent evidence indicates that rapid up-regulation of glucose transport in jejunal enterocytes occurs by a change in the abundance of SGLT1 in the apical membrane (6, 11).

The transcellular transport of aldoses (D-glucose, 3-O-MG, and D-galactose) across the absorptive epithelium (enterocytes) involves entry across the BBM using the Na+-dependent transporter (SGLT1) (12, 13) followed by exit across the BLM via a Na+-independent transporter (GLUT2) (14). D-Fructose, a ketose, enters the enterocyte using a different carrier, a Na+- independent transporter (GLUT5) (15), but exits using the same transporter (GLUT2) as the aldoses in the BLM (15) (Fig. 1). Thus, by applying this model and monitoring both aldose and ketose absorption the results would allow for a better understanding of the specificity and locus of action of CCK-8 on hexose absorption. The dually perfused jejunal preparation has been useful in determining the effects of hormones responsible for regulating carbohydrate absorption (4, 7), the locus of action of inhibitors of specific hexose transporters by compartmental analysis (16), and the simultaneous measurement of aldose and ketose absorption (17). To establish if the CCK-8-induced decrease in hexose absorption involves changing the number of transporters in the BBM we measured in each tissue the rate of hexose absorbed over time, using the dually perfused jejunal and then determined the abundance of SGLT1 in the BBM at the specific transport rates at the same perfusion times.

In this report we demonstrate that CCK-8 specifically inhibits aldose absorption by decreasing the abundance of the SGLT1 protein located in the BBM of the rat small intestine. Our results establish that the rate at which the jejunal absorbs aldoses is subjected to rapid and specific enteric peptide control of the rate of entry across the BBM.

EXPERIMENTAL PROCEDURES

Dually Perfused in Situ Jejunum—Male Sprague-Dawley rats (200–350 g) were supplied by Taconic Farms, Germantown, NY. The rats were fed a standard chow diet (Purina PMI Rodent Food) and water ad libitum. Before the start of the experiment, food was withdrawn for approximately 24 h to minimize intestinal luminal contents during surgery. The study was approved by the Health Sciences Animal Welfare Committee from the Faculty of Medicine. All rats were anesthetized prior to surgery using sodium pentobarbital given by intraperitoneal injection (60 mg/kg body weight) and placed on a heated (37 °C) surgical table. The techniques and apparatus used in these experiments...
were similar to those described previously (4, 17). After performing a laparotomy, the blood supply to the spleen, rectum, colon, stomach, and ileum were tied off and the tissues removed; the vasculature to the pancreas and duodenum were also ligated. A 35-cm segment of jejunum, starting 5 cm distal from the ligament of Trietz, was isolated and the luminal contents removed by gently flushing with 20 ml of warm saline (0.9%), and the jejunum was cannulated at both ends. The lumen was perfused with a Krebs-bicarbonate saline solution (120 mM NaCl, 4 mM KCl, 2.5 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 1 mM CaCl₂) using a GILSON Minipuls 2 pump (Mandel Scientific Ltd). The solution, containing 5 mM 3-O-MG or 5 mM 3-O-MG and 5 mM D-fructose, was maintained at 37 °C and gassed with 95% O₂, 5% CO₂. Isotopically labeled hexoses, 10 μCi of either 3-O-methyl-D-[1-3H]glucose, or both 3-O-methyl-D-[1-3H]glucose and D-[U-14C]fructose (Amer sham Canada Ltd.), were added to the luminal circuit immediately after portal vein cannulation. The single-pass luminal circuit was perfused at a flow rate of 1.6 ml/min, and the solution was segmented by 95% O₂, 5% CO₂ gas bubbles. The gas bubbles were introduced into the luminal perfusate through a Y piece at a flow rate which ensured that bubbles occupied the diameter of the perfusion tube. This not only exposed the tissue to a saturating gas partial pressure, but also helped to mix the solution in the tissue lumen. After a single-pass through the segment of jejunum the luminal perfusate was discarded. The aorta, proximal to the superior mesenteric artery, was ligated just prior to insertion of a cannula into the superior mesenteric artery. The single-pass vascular circuit was perfused at a rate of 1.6 ml/min with fresh Krebs-bicarbonate saline solution, containing 5 mM D-glucose, 0.034 mM streptomycin sulfate, 5 mM L-glutamine, 1120 USP units heparin, and 10% w/v Ficoll 70 (Sigma-Aldrich Canada Ltd.) as a plasma expander, which was maintained at 37 °C and gassed with 95% O₂, 5% CO₂, maintaining the pH at 7.4. Once the vascular circuit was established, the rat was euthanized and the vascular perfusate was collected via a cannula in the hepatic portal vein. CCK-8 was added to the vascular perfusate at final concentrations described in the appropriate figure legend. The effluent was collected continuously for up to 80 min and the vascular effluent was collected over a 70-min period via a cannula in the portal vein. The solid line (>) represents the rate of appearance of labeled 3-O-MG in the vascular effluent. The dashed line (□) represents the appearance of labeled D-fructose in the vascular effluent. The solid horizontal black bar represents 8 pmol CCK-8 in the vascular perfusate.

Preparation of Brush-border Membrane Vesicles—The frozen mucosal scrapings, taken from tissue used to measure hexose absorption after 25 min of perfusion with or without CCK-8 present in the vascular influse, were thawed at room temperature and then placed in 40 ml of ice-cold mannitol/Tris buffer (300 mM mannitol, 5 mM EGTA, 12 mM Tris-HCl, pH 7.4, 0.1 mM phenylmethylsulfonyl fluoride). The tissue was homogenized with a Polytron homogenizer (setting 5) for 2 min before addition of magnesium chloride to a final concentration of 12 mM. After stirring the solution on ice for 15 min the solution was centrifuged at 3,000 × g (Sorval RC5C) for 15 min to remove debris. The supernatant was further centrifuged at 37,000 × g for 30 min and the pellet homogenized in a mannitol/Tris buffer (150 mM mannitol, 2.5 mM EGTA, 6 mM Tris-HCl, pH 7.4, 0.05 mM phenylmethylsulfonyl fluoride) with a glass homogenizer before further addition of magnesium chloride (12 mM). After stirring on ice the centrifugation was repeated as before and the pellet was then washed with 300 mM mannitol, 5 mM Tris-HCl, pH 7.4, before repelleting. This vesicle preparation was diluted in 300 mM mannitol, 5 mM Tris-HCl, pH 7.4, to an appropriate protein concentration, usually 8 mg/ml.

Western Blotting—Brush-border membrane vesicles (see above) (40 μg) from control and CCK-8 treated tissue were solubilized in Laemmli sample buffer and run on a 10% sodium dodecyl sulfate-polyacrylamide gel using a Mini-PROTEAN II cell (Bio-Rad, Canada). The proteins were transferred onto nitrocellulose membrane (Millipore) by electrotransfer for 90 min, at 4 °C, using the Mini Trans-Blot Cell (Bio-Rad, Canada). Blocking of the membrane was carried out in 3% nonfat milk in PBST (0.05% Tween 20, phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 4.3 mM NaHCO₃), pH 7.4, for 1 h. The blots were incubated in 3% nonfat dry milk in PBST with 1:1,000 rabbit polyclonal antibody to rat SGLT1 (Chemicon International Inc., Temecula, CA) overnight at 4 °C. The membrane was washed three times in 3% nonfat dry milk, PBST for 15 min. The nitrocellulose membrane...
FIG. 3. CCK-8 decreases the amount of 3-O-MG entering the enterocytes. The jejunal lumen was perfused with Krebs bicarbonate saline, segmented with carbogen gas bubbles, at a flow rate of 1.6 ml/min. ³H-labeled 3-O-MG was added to the luminal circuit (containing 5 mM 3-O-MG) within 3 min after cannulation of the portal vein. The vascular bed was perfused with a Ficoll-based Krebs solution as described under "Experimental Procedures," at the same flow rate as in the lumen. The vascular effluent was collected over a 38-min period via a cannula in the portal vein. The solid line (○) represents the rate of appearance of labeled 3-O-MG in the vascular effluent. The dashed line (●) represents appearance of labeled 3-O-MG in the vascular effluent with 8 pm CCK-8 in the vascular infusate. The horizontal solid black bar represents the lumenal washout (0 mM 3-O-MG) in the presence of 5 mM mannitol. Data points represent the mean rate of appearance of 3-O-MG expressed as absorption in micromoles/g dry weight/h ± S.E., n = 3 (p < 0.05).

was then incubated with a secondary antibody, anti-rabbit IgG coupled to horseradish peroxidase diluted 1:2,000 in 3% nonfat dry milk, PBST for 1 h. Three subsequent washes followed as described above. Finally, the membrane was treated with the ECL detection solution (Amersham Canada Ltd.) before exposing the Kodak XAR-5 film with an intensifying screen from 1 to 4 min. One distinct band was detected by this method with an apparent molecular mass of 71 kDa.

Immunoblot Quantitative Analysis—Immunoblots were scanned with a Scanjet 4C flatbed scanner (Hewlett Packard, Palo Alto, CA), calibrated with a Kodak gray scale. Scanned images were quantified using NIH Image 1.60 software. Results are reported as percentage from protein abundance at steady state rate after 25 min of perfusion with 5 mM 3-O-MG. Statistical analysis was performed using Student’s t test.

RESULTS

To determine the specificity of CCK-8’s actions and to identify which of the transporter proteins are involved in the CCK-induced inhibition of intestinal hexose absorption, both the rates of D-fructose and 3-O-MG absorption were measured simultaneously in dually perfused jejunum (Fig. 2). After the steady-state rate of 3-O-MG transport was achieved (12 min), the rate significantly diminished after CCK-8, 8 pm (the maximum inhibitory concentration) (4) was added to the vascular infusate. The steady-state rate of 3-O-MG in this preparation is normally maintained for a minimum of 90 min. in the absence of vascular CCK-8 (4). The steady-state rate of fructose absorption was slower than that of 3-O-MG, and was achieved more slowly (23 min). Also, when CCK-8 was added to the vascular perfusate fructose transport was unaffected (Fig. 2).

The fructose data indicates that the locus of CCK-8 action is specific for SGLT1 in the BBM (refer to Fig. 1) so we used compartmental analysis of six separate washout studies of 3-O-MG from the small intestine to confirm this observation. Addition of CCK-8 (8 pm) to the vascular perfusate caused a significant decline in the steady-state transport rate (Fig. 3), and analysis of the washout using double exponential decay (Enzfitter software, Elsevier) showed CCK-8 significantly decreased the pool size, Q₀₁ within the epithelium. In contrast the second pool, Q₀₂, and the rate constants K₁ and K₂ were not significantly reduced by CCK-8 compared with control conditions (steady-state rate) (Fig. 4, A and B, and Table I).

To determine the mechanism involved in the CCK-8-induced decrease in SGLT1-mediated transport, we monitored 3-O-MG absorption and then measured the abundance of SGLT1 protein in tissue after a 25-min perfusion either with or without CCK-8 (8 pm) present in the vascular infusate. A significant decrease in SGLT1 abundance (Fig. 5B) caused by the addition of CCK-8 (8 pm) to the vascular infusate was shown to parallel a decrease in the 3-O-MG absorption (Fig. 5A), which occurred within 25 min of CCK-8 addition to the vascular circuit. To help make the comparison and determine statistical significance the data were converted to a percent of control values, the change in transport rate and SGLT1 abundance induced by the presence of CCK-8 (8 pm) was 25.2 ± 4 and 36 ± 6.7, respectively, and the changes induced by CCK-8 were not significantly different from each other (Fig. 5C).
DISCUSSION

Slowing the rate at which D-glucose enters the systemic circulation would improve the ability of the other glucose homeostatic systems to handle the substrate load and therefore contribute to the normalization of the plasma glucose concentration. The results from the simultaneous fructose and D-3-O-MG absorption study suggest that GLUT5 in the BBM and GLUT2 in the BLM of the enterocyte, both of which transport fructose, are not affected by CCK-8 and supports the view that CCK acts specifically on SGLT1 (refer to Fig. 1). The only alternative explanation for this data could be that CCK selectively changes the affinity of GLUT2 in the BLM for aldoses and not fructose. What is also noteworthy from the hexose absorption measurements is the lower rate of fructose absorption (2.7-fold slower) compared with that of 3-O-MG (Fig. 2). This slower fructose absorption in rat jejunum is similar to that reported by Holloway and Parsons (18) using a similar dual perfusion technique. The slower rate of fructose absorption we observed could be explained by the different transporters employed in aldose and ketose absorption. D-Fructose entry into the enterocyte is not driven by the Na⁺ gradient, but is apparently concentration driven, a consequence of the metabolism that occurs in the enterocytes. It is likely that the metabolic fate of fructose is one of the primary determinants in regulating absorption and therefore the rate of D-fructose absorption depends significantly on the animal’s ability to metabolize fructose (19). In order to support our hypothesis that CCK-8 acts specifically on the SGLT1 transporter, indicated by the fructose absorption studies, we used compartmental analysis of 3-O-MG washout in the dually perfused jejunum, which showed a significant decrease in the tissue pool size. This method was used previously to indicate the site of the rate-limiting step for hexose and amino acid transport across the enterocyte (16, 20, 21) and our data parallel those of Boyd and Parsons (16), who showed that phlorizin, which is known to act specifically on SGLT1, significantly decreased the Qₐ without affecting the washout rate constant. In these experiments CCK-8 also significantly reduced the tissue pool size of 3-O-MG which could only occur if entry across the BBM was reduced, or exit across the BLM was increased. If anything, CCK-8 slowed the exit as measured by the rate constant K₂, although the effect was not statistically significant. Therefore, the reduced tissue size most likely results from a decreased uptake across the BBM. Taken together, the compartmental analysis data and the unaffected fructose absorption indicate that CCK’s action is to reduce the entry of aldoses across the BBM, i.e. substrates specific for SGLT1.

The immunoblots showing a decrease in SGLT1 indicate that CCK is involved in a rapid post translational event which lowers SGLT1 abundance. This could mean that CCK regulates SGLT1 transporter translocation in a manner similar to that for GLUT1 and GLUT4, which occurs in fat and muscle tissue (22). Additional evidence which supports this type of regulation in the intestine includes the fact that changes occur in the surface area of enterocytes when glucose absorption is increased with epidermal growth factor (23). Furthermore, GLP-2 and epinephrine have also been shown to increase SGLT1 abundance in this tissue (6, 11). There is also some evidence, using Xenopus oocytes expressing SGLT1, that protein kinase A and C modulate exocytosis and endocytosis, respectively, of vesicles containing SGLT1 (24). Because the physiological effect of CCK occurs at the BBM, when the peptide is added to the vascular circuit, it is likely that this action is mediated by a cytosolic second messenger and does not occur directly through a receptor mediated endo or exocytosis. The decrease in SGLT1 abundance could result from a reduced rate of insertion of SGLT1 into the BBM, from an increased rate of

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**TABLE I**

Effect of CCK-8 on pool sizes and rates of washout of 3-O-MG in the dually perfused jejunum

| Sample                          | Pool size, Qₐ | Rate constant, K₁ | Pool size, Qₐ | Rate constant, K₂ |
|---------------------------------|---------------|------------------|---------------|------------------|
| Control (without CCK-8 in vascular circuit) | 223 ± 4.8     | 0.66 ± 0.14      | 8.1 ± 2.4     | 0.06 ± 0.07      |
| CCK-8 (8 pM CCK-8 included in vascular circuit) | 90 ± 3.4      | 0.34 ± 0.07      | 3.2 ± 1.1     | 0.02 ± 0.01      |

*p < 0.05 from control.

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**FIG. 5.** CCK-8 decreases 3-O-MG absorption in parallel to a reduction in SGLT1 abundance in the brush-border membrane. Absorption of labeled 3-O-MG was measured for 25 min using the dually perfused preparation (panel A) as described under “Experimental Procedures.” After collecting the vascular effluent the jejunal segment was excised from the animal, rinsed with ice-cold Krebs solution, opened longitudinally, and the mucosa scraped off using a glass slide. The mucosal scrapings were then excised from the animal, rinsed with ice-cold Krebs solution, and immediately snap-frozen in liquid nitrogen. The mucosal scrapings were subsequently homogenized and immunodetected using an SGLT1 antibody (panel B, a, protein density after 25 min of lumenal perfusion with 5 mM 3-O-MG (steady-state rate); b, c, d, and e (each a separate experiment, n = 4) after 25 min with 8 pM CCK-8 in the vascular circuit and 5 mM 3-O-MG in the lumenal perfusate. In panel C the black columns represent protein abundance (arbitrary units) and the white columns represent the absorption (% of control). Values represent mean ± S.E. where 100% represents the protein abundance and the absorption rate at steady-state without CCK-8 present, n = 4 (*p < 0.05).
removal from the BBM, or by decreasing a recycling step. However, the decrease in SGLT1 abundance indicates that the action is not likely to be mediated through the proposed regulatory subunit of SGLT1 (RS1), unless RS1 acts as a chaperone (25). Also, evidence has suggested that regulation of SGLT1 in rat jejunum is mediated through a protein kinase A phosphorylation (11), however there are no apparent protein kinase A consensus phosphorylation sites on the rat SGLT1 (24). This suggests that phosphorylation would likely involve another component involved in insertion or removal of SGLT1 from the BBM, as mentioned above.

The establishment of a rapid negative feedback pathway involving CCK for controlling hexose absorption in the small intestine extends the role of the tissue in glucose homeostasis. Instead of an immediate and rapid absorption of all SGLT1 specific substrates, there is a slowing in the rate of transfer while the meal is passing along the small intestine. This would allow for a more gradual introduction of glucose into the body, and would give the other tissues more time to handle this nutrient and serve to help maintain a steady plasma glucose concentration.

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