Digestive Diseases and Sciences, Vol. 45, No. 11 (November 2000), pp. 2122–2126

Noninvasive *In vivo* Analysis of Human Small Intestinal Paracellular Absorption:

Regulation by Na^+–Glucose Cotransport

JERROLD R. TURNER, MD, PhD, DAVID E. COHEN, MD, PhD, RANDALL J. MRSNY, PhD, and JAMES L. MADARA, MD

Activation of intestinal Na^+–glucose cotransport increases paracellular movement of inert tracers in cultured monolayers, isolated rodent intestinal mucosae, and in rodents *in vivo*. However, not all studies have demonstrated comparable effects on human intestinal paracellular absorption. We sought to assess the effects of Na^+–glucose cotransport on paracellular absorption in human beings using a simple noninvasive assay. Study subjects drank six 200-ml doses of test solution, composed of 0.8% w/v creatinine (sufficient to overwhelm endogenous creatinine) in 277 mM glucose or mannitol and urine was collected. Intestinal creatinine absorption is paracellular. Once absorbed, creatinine is cleared into the urine. Therefore, urinary creatinine recovery reflects intestinal paracellular creatinine absorption. Total urinary creatinine recovery was 55% ± 4% of creatinine ingested with glucose and 38% ± 9% of creatinine ingested with mannitol ($p < 0.001$). Thus, intestinal paracellular absorption of creatinine is increased by the presence of luminal glucose. Our results are consistent with *in vivo* human regulation of mucosal permeability by Na^+–glucose cotransport.

KEY WORDS: paracellular absorption; solvent drag; nutrient absorption; water absorption; Na^+–glucose cotransport; tight junction regulation.

Small intestinal mucosal nutrient transport occurs primarily via active epithelial transport. In the case of glucose, the single most abundant actively transported nutrient in human diets, active transport occurs at the apical, or luminal, surface via the Na^+–glucose cotransporter, SGLT1 (1, 2). Glucose then crosses the enterocyte basolateral membrane through the facilitated glucose exchanger GLUT2. In the past 10 years, paracellular nutrient uptake by the paracellular pathway has been proposed and demonstrated in rodents, both in isolated intestinal mucosae and *in vivo* (3–9). Although this result has not been shown uniformly, it is clear that some studies that did not demonstrate enhanced paracellular absorption in the presence of active Na^+–glucose cotransport used methods that produced inadequate paracellular fluid absorption (10–17). Since the proposed mechanism of solvent drag requires paracellular water absorption, these studies cannot be considered valid refutations of the effects of Na^+–glucose cotransport on paracellular movement of small molecules. Thus, the physiological significance of paracellular nutrient uptake remains controversial (10–12).

Studies of paracellular absorption have been primarily performed in rodents. When paracellular

Manuscript received December 22, 1999; accepted March 12, 2000.

From the Department of Pathology, Wayne State University, Detroit, Michigan; Liver Research Center, Albert Einstein College of Medicine, Bronx, New York; Department of Pharmaceutical Research and Development, Genentech Inc., South San Francisco, California; and Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia.

Address for reprint requests: Dr. Jerrold R. Turner, Wayne State University School of Medicine, 540 E. Canfield, 9245 Scott Hall, Detroit, Michigan 48201. e-mail: jturner@med.wayne.edu

0163-2116/00/1100-2122$18.00/0 © 2000 Plenum Publishing Corporation

probes are delivered with sufficient water to drive solvent drag, these have generally been successful in demonstrating increased paracellular flux after Na^+–glucose cotransport, although some studies have not shown this effect (6, 8, 9, 11, 12, 17, 18). In contrast, studies in humans, using multilumen catheters and functionally isolated perfused loops have consistently failed to demonstrate increased paracellular flux after Na^+–glucose cotransport (15, 16). As noted above, a likely explanation for this discrepancy is that insufficient absorption occurs in the isolated loops over the short segments evaluated (10–12). Thus, we designed an *in vivo* method for analyzing paracellular absorption over extended lengths of human small intestine. We used creatinine, a classic marker of the extracellular space. A similar approach has been used previously to demonstrate *in vivo* regulation of intestinal paracellular absorption in normal unanesthetized rats (5). Since creatinine is absorbed paracellularly in the intestine and freely filtered at the glomerulus, urinary recovery of creatinine allows indirect assessment paracellular absorption. Using this approach, we observed a 45% increase in creatinine recovery in the presence vs absence of active Na^+–glucose transport in human subjects.

MATERIALS AND METHODS

Subjects. This study was approved by the institutional review board. Six healthy human volunteers (three male, three female) were recruited. Each underwent a complete history and physical examination and subjects with gastrointestinal, circulatory, or renal disease or dysfunction were excluded. Subjects taking NSAIDs or other agents that might alter gastrointestinal permeability were also excluded, as were those with a family history of inflammatory bowel disease. Each subject had normal CBC and normal serum chemistries. Additionally, normal renal function was verified in each by serum creatinine and BUN measurements and urinalysis, including urinary creatinine measurement. Subjects were asked to refrain from alcohol on evenings prior to study dates and to partake of a breakfast composed of no more than one glass of orange juice, two slices of toast with jam and/or butter, and a cup of coffee the following morning. Subjects were then instructed to eat or drink nothing for 2 hr prior to ingestion of the first test solution. During the study interval (6 hr), subjects limited their intake to the test solutions only.

Solutions. Study solutions were composed of 277 mM carbohydrate and 0.8% (w/v) creatinine. The carbohydrate used was either glucose or, as a non-Na^+-cotransported control, mannitol. Solutions were prepared in bulk and then separated into 200-ml portions for administration during the study. Representative aliquots were retained for quantitative analysis.

Study Design. Subjects were studied using a crossover protocol. Subjects were blinded as to which solution they ingested. Each subject drank one creatinine-containing solution (glucose or mannitol) on the first test day and the other test solution on the second day of testing (two days later). After voiding (this specimen was discarded), subjects drank 200 ml of a test solution. Five additional 200-ml portions of the same test solution were taken at 45-min intervals, for a total of 1200 ml. Urine was collected at intervals for 6 hr after ingestion of the first test solution. Subjects were asked to void at the completion of the study (6 hr after the first solution was ingested). Urine was collected into sterile containers and labeled with the time after study initiation at which the sample was obtained. The volume was measured and NaN_3 was added to 0.05% to prevent bacterial growth. The urine specimens were then stored at −20°C until creatinine was assayed.

Creatinine Assay. Urinary creatinine was measured using the DuPont ACA analyzer based on the chromogenic reaction following addition of picrate in the presence of NaOH. The absorbance was measured at 510 nm and creatinine concentration calculated from a standard curve. Urine specimens were diluted 10- to 100-fold so that all measurements were performed within the linear range of the assay.

Statistical Analysis. The interval and cumulative urinary creatinine recovery was determined for each subject. Each subject's urinary creatinine recovery after ingestion with glucose vs mannitol was then compared by paired *t* test.

RESULTS

We and others have demonstrated that activation of intestinal Na^+–glucose cotransport increases paracellular movement of inert tracers in cultured monolayers, isolated rodent intestinal mucosae, and in rodents *in vivo* (3, 5, 8, 9, 11, 19, 20). In order to assess changes in paracellular absorption in humans, we sought to design an experimental approach in which glucose absorption would be complete (10), surgical manipulation and anesthesia were unnecessary (21), and a significant intestinal length was assayed (10). We also sought to use molecular probes of appropriate size to allow detection of changes in paracellular absorption. Both the radius (3.2 Å) and molecular weight (113.12 g) of creatinine are similar to those of glucose (3.7 Å, 180.16 g) and other small nutrients. Additionally, Pappenheimer and Reiss have shown that luminal glucose increases absorption of creatinine by 2.9-fold in rodents (5). Thus, we concluded that creatinine would be an appropriately sized probe to measure glucose-induced increases in paracellular permeability in humans. Creatinine is not absorbed transcellularly and, once absorbed, is freely filtered at the glomerulus. Thus, urinary recovery of creatinine is a simple indirect measure of intestinal paracellular creatinine absorption. Subjects were evaluated for normal serum and urinary creatinine and normal renal function in order to prevent interference from

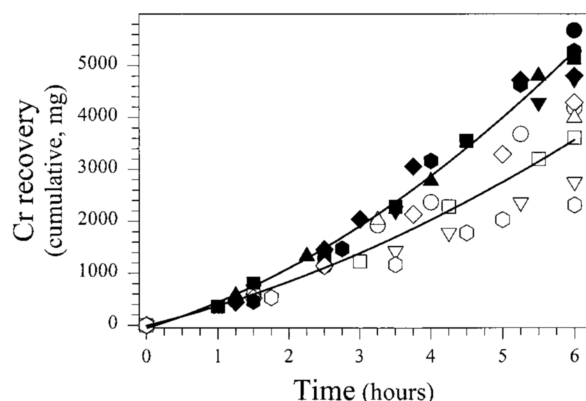

Fig 1. Urinary creatinine recovery is increased by Na^+–glucose cotransport. Cumulative urinary creatinine recovery is plotted as a function of time. Creatinine was ingested at the beginning of the study and at 5 additional time points at 45-min intervals. Urine was collected until 6 hr after the initial ingestion. Filled symbols represent creatinine recovery after ingestion in a glucose-containing test solution, while open symbols represent creatinine recovery after ingestion in a mannitol-containing test solution. Each study subject is represented by a unique symbol shape (filled or open).

endogenous creatinine. Subjects ingested more than 9 g of creatinine during the course of this study. Thus, absorption of even a fraction of the ingested creatinine would be sufficient to render endogenous creatinine insignificant. In order to minimize regulation of intestinal permeability by dietary carbohydrate, subjects were instructed to partake of a maximally defined breakfast and fast for at least 2 hr prior to the initiation of the study. Oral intake was limited to the test solutions during the 6 hr of the study.

When ingested with glucose, urinary creatinine was recovered at an average rate of 14.2 ± 0.47 mg/min. In contrast, urinary creatinine was recovered at an average rate of 10.3 ± 1.1 mg/min when ingested with mannitol ($P < 0.01$). As shown in Figure 1, total urinary creatinine recovery ranged from 51% to 61% of creatinine ingested with glucose (mean 54% ± 2%). When ingested with mannitol, total urinary creatinine recovery ranged from 25% to 46% of creatinine ingested with glucose (mean 38% ± 4%, $P < 0.01$). As was true of the group comparisons, creatinine recovery was greater when ingested with glucose than with mannitol for each individual subject (Table 1).

As anticipated, total urinary volume was increased when glucose solutions were ingested (mean 1140 ± 170 ml, range 400–1460 ml) relative to when mannitol solutions were ingested (mean 420 ± 70 ml, range 180–570 ml, $P < 0.01$). Nonetheless, since no subject complained of significant diarrhea, the entire ingested volume of 1200 ml was absorbed and able to support paracellular movement of creatinine.

TABLE 1. TOTAL URINARY CREATININE RECOVERY AFTER 6 HOURS

Subject	*Glucose (mg)*	*Mannitol (mg)*
1	4800	3990
2	5150	3610
3	4800	4280
4	5280	2310
5	4740	2760
6	5680	4180
Average ± SE	5080 ± 170	3520 ± 360

DISCUSSION

The regulation of paracellular absorption in the small intestine has been debated since its initial description in 1987 (3–5). At that time, Pappenheimer, Madara, and Reiss demonstrated regulation of transmucosal impedance, paracellular flux, and tight junction ultrastructural morphology as consequences of Na^+–nutrient cotransport in isolated small intestinal mucosa. They also demonstrated the ability of a simple 5% glucose solution to enhance intestinal creatinine absorption, relative to water, *in vivo* in rodents (5). Subsequent studies have demonstrated a molecular basis for this proposed regulation using a reductionist cell culture model in which transit rate and vascular countercurrent multiplier effects were eliminated (20). However, the conclusion that luminal Na^+–glucose cotransport augments passive paracellular flux of small molecules continues to be controversial (3–11, 13–17, 19, 21–28).

Much of mucosal nutrient absorption occurs via active epithelial transport. In the case of glucose, active transport occurs at the apical, or luminal, surface via the Na^+–glucose cotransporter, SGLT1. Glucose then crosses the enterocyte basolateral membrane through the facilitated glucose exchanger GLUT2. The increased paracellular nutrient uptake described above is thought to occur as a result of stimuli triggered by transcellular glucose transport and water flow, ie, via solvent drag (4, 5, 19). Thus, paracellular water flow is the driving force for Na^+–glucose cotransport-associated increases in paracellular absorption of small solutes (4–6, 19).

Studies of paracellular uptake following activation of Na^+–glucose cotransport have been primarily performed in rodents (3–9, 19, 28). When paracellular probes are delivered with sufficient water to drive solvent drag, these have generally been successful in

demonstrating increased paracellular flux after Na^+–glucose cotransport. When a similar approach to that used in this study was used to demonstrate *in vivo* regulation of intestinal permeability in normal unanesthetized rats and rabbits, recovery of creatinine in the urine was markedly enhanced by glucose (5, 19). For rabbits, 36.6% ± 3.5% of ingested creatinine was recovered in the urine when the creatinine was mixed with water, but 53.4% ± 4.5% was recovered when the creatinine was mixed with glucose-containing water (19). Similarly, in rats only 37% ± 2.3% of an oral creatinine load was recovered in the urine when administered with water, but 53% ± 2.4% of creatinine was recovered in the urine when the water contained glucose (5). Remarkably, these numbers are nearly identical to each other and to the 38% ± 9% and 55% ± 4% urinary creatinine recoveries we noted in humans who ingested creatinine with mannitol or glucose, respectively.

In contrast, human studies, using multilumen catheters and functionally isolated perfused loops have failed to demonstrate increases in paracellular absorption after Na^+–glucose cotransport (15, 16), as have studies of isolated loops in animals (13). One such study concluded that paracellular absorption of D-glucose is trivial in humans (16). However, as analyzed in detail elsewhere (10), paracellular water absorption was small and likely insufficient to demonstrate paracellular nutrient absorption. Indeed, a follow-up study documented a 129% increase in paracellular absorption of the inert marker L-xylose when Na^+–glucose cotransport was activated (15). As would be predicted, this study also demonstrated that this increased paracellular absorption required water absorption (15). Similarly, paracellular absorption of glucose was not documented in rats fed tracers with dry chow (17). Again, the probable explanation for the discordance with results of previous studies is likely the absence of paracellular water absorption (11). Most recently, a study using dogs with surgically created Thiry-Vella loops examined the contribution of paracellular transport to glucose absorption (13). Fractional water absorption was only 5% ± 3% and 7% ± 1% for solutions with 1 or 50 mM D-glucose, respectively. Nonetheless, paracellular D-glucose absorption increased from 9% ± 4% to 24% ± 10% of the total D-glucose absorbed (13). While this difference did not reach statistical significance (13), it is certainly worthy of note, as it reflects a 35-fold increase in passive D-glucose absorption (from 0.28 to 10 μmol/min) despite similar rates of water absorption (0.235 vs 0.329 ml/min). Thus, in contrast to the conclusions reached by Lane et al (13), this study may have demonstrated increases in paracellular D-glucose absorption that are triggered by transcellular D-glucose absorption.

Thus, while some studies have failed to demonstrate increases in paracellular absorption after stimulation of transcellular Na^+–glucose cotransport, there are logical explanations for these differences. In studies designed to allow absorption of water sufficient to support solvent drag, paracellular movement of small solutes has been easily demonstrated. Our study represents the first study of paracellular solute absorption in humans performed using a noninvasive method. While these data do not provide analysis of effective pore radii, they do support the solvent drag model of paracellular nutrient absorption first proposed in 1987 (3–5). Moreover, these studies document the utility of creatinine as a simple and easily assayed probe of intestinal paracellular absorption in humans. Given the wide availability of quantitative creatinine assays, the simple ingestion of a creatinine-containing solution followed by urinary recovery may be a useful clinical tool for the study of intestinal disease.

REFERENCES

1. Hediger MA, Coady MJ, Ikeda TS, Wright EM: Expression cloning and cDNA sequencing of the Na^+/glucose cotransporter. Nature 330:379–381, 1987
2. Turk E, Zabel B, Mundlos S, Dyer J, Wright EM: Glucose/galactose malabsorption caused by a defect in the Na^+/glucose cotransporter. Nature 350:354–356, 1991
3. Madara JL, Pappenheimer JR: Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. J Membr Biol 100:149–164, 1987
4. Pappenheimer JR: Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters. J Membr Biol 100:137–148, 1987
5. Pappenheimer JR, Reiss KZ: Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. J Membr Biol 100:123–136, 1987
6. Pappenheimer JR: On the coupling of membrane digestion with intestinal absorption of sugars and amino acids. Am J Physiol 265:G409–G417, 1993
7. Atisook K, Carlson S, Madara JL: Effects of phlorizin and sodium on glucose-elicited alterations of cell junctions in intestinal epithelia. Am J Physiol 258:C77–C85, 1990
8. Atisook K, Madara JL: An oligopeptide permeates intestinal tight junctions at glucose-elicited dilatations. Implications for oligopeptide absorption. Gastroenterology 100:719–724, 1991
9. Sadowski DC, Meddings JB: Luminal nutrients alter tight-junction permeability in the rat jejunum: An *in vivo* perfusion model. Can J Physiol Pharmacol 71:835–839, 1993
10. Madara JL: Sodium–glucose cotransport and epithelial permeability. Gastroenterology 107:319–320, 1994
11. Turner JR, Madara JL: Physiological regulation of intestinal

epithelial tight junctions as a consequence of Na^+–coupled nutrient transport. Gastroenterology 109:1391–1396, 1995
12. Soergel KH: Showdown at the tight junction. Gastroenterology 105:1247–1250, 1993
13. Lane JS, Whang EE, Rigberg DA, Hines OJ, Kwan D, Zinner MJ, McFadden DW, Diamond J, Ashley SW: Paracellular glucose transport plays a minor role in the unanesthetized dog. Am J Physiol 276:G789–G794, 1999
14. Uhing MR, Kimura RE: Active transport of 3-O-methyl glucose by the small intestine in chronically catheterized rats. J Clin Invest 95:2799–2805, 1995
15. Fine KD, Santa Ana CA, Porter JL, Fordtran JS: Mechanism by which glucose stimulates the passive absorption of small solutes by the human jejunum *in vivo*. Gastroenterology 107:389–395, 1994
16. Fine KD, Santa Ana CA, Porter JL, Fordtran JS: Effect of D-glucose on intestinal permeability and its passive absorption in human small intestine *in vivo*. Gastroenterology 105:1117–1125, 1993
17. Schwartz RM, Furner JK, Levitt MD: Paracellular intestinal transport of six-carbon sugars is negligible in the rat. Gastroenterology 1995
18. Perez M, Barber A, Ponz F: *In vivo* increase of passive intestinal absorption by cotransporter activation in rat. Rev Esp Fisiol 49:259–264, 1993
19. Pappenheimer JR: Paracellular intestinal absorption of glucose, creatinine, and mannitol in normal animals: relation to body size. Am J Physiol 259:G290–G299, 1990
20. Turner JR, Rill BK, Carlson SL, Carnes D, Kerner R, Mrsny RJ, Madara JL: Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. Am J Physiol 273:C1378–C1385, 1997
21. Uhing MR, Kimura RE: The effect of surgical bowel manipulation and anesthesia on intestinal glucose absorption in rats. J Clin Invest 95:2790–2798, 1995
22. Madara JL: Tight junction dymnamics: Is paracellular transport regulated. Cell 53:497–498, 1992
23. Madara JL, Carlson S: Supraphysiologic L-tryptophan elicits cytoskeletal and macromolecular permeability alterations in hamster small intestinal epithelium in vitro. J Clin Invest 87:454–462, 1991
24. Pappenheimer JR, Volpp K: Transmucosal impedance of small intestine: correlation with transport of sugars and amino acids. Am J Physiol 263:C480–C493, 1992
25. Pappenheimer JR, Dahl CE, Karnovsky ML, Maggio JE: Intestinal absorption and excretion of octapeptides composed of D amino acids. Proc Natl Acad Sci USA 91:1942–1945, 1994
26. Ballard ST, Hunter JH, Taylor AE: Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. Annu Rev Nutr 15:35–55, 1995
27. Madara JL: Regulation of the movement of solutes across tight junctions. Annu Rev Physiol 60:143–159, 1998
28. Jodal M, Fihn B-M, Sjøqvist A: Effect of glucose on passive transport of extracellular probes across the rat small intestinal epithelium *in vivo*. Gastroenterology 107:A241, 1994