SCFA transport in rat duodenum

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Kaji I, Iwanaga T, Watanabe M, Guth PH, Engel E, Kaunitz JD, Akiba Y. SCFA transport in rat duodenum. Am J Physiol Gastrointest Liver Physiol 308: G188–G197, 2015. First published November 13, 2014; doi:10.1152/ajpgi.00298.2014.—Bacterial or ingested food-derived short-chain fatty acids (SCFAs) are present in the duodenal lumen. Acetate, the most abundant SCFA in the foregut lumen, is absorbed immediately after ingestion, although the mechanism by which this absorption occurs is not fully understood. We investigated the distribution and function of candidate SCFA transporters in rat duodenum. The Na+-coupled monocarboxylate transporter-1 (SMCT1) was localized to the brush border, whereas the pH-dependent monocarboxylate transporter (MCT) 1 and MCT4 were localized to the duodencyte basolateral membrane. In Ussing chambered duodenal mucosa, luminal acetate dose-dependently increased short-circuit current (Isc) in the presence of serosal bumetanide and indomethacin with a luminal Na+-dependent, ouabain-sensitive mechanism. The Isc response was inhibited dose-dependently by the SMCT1 nonsubstrate inhibitor ibuprofen, consistent with net electrogenic absorption of acetate via SMCT1. Other SCFAs and lactate also increased Isc. Furthermore, duodenal loop perfusion of acetate increased portal venous acetate concentration, inhibited by coperfusion of ibuprofen or a MCT inhibitor. Luminal acetate perfusion increased duodenal HCO3− secretion via capsaicin-sensitive afferent nerve activation and cyclooxygenase activity, consistent with absorption-mediated HCO3− secretion. These results suggest that absorption of luminal SCFAs via SMCT1 and MCTs increases duodenal HCO3− secretion. In addition to SCFA sensing via free fatty acid receptors, the presence of rapid duodenal SCFA absorption may be important for the suppression of luminal bacterial colonization and implicated in the generation of functional dyspepsia due to bacterial overgrowth.

Acetate; monocarboxylate transporter; sodium-coupled monocarboxylate transporter; short-chain fatty acid

SHORT-CHAIN FATTY ACIDS (SCFAs) such as acetic (2-carbon), propionic (3-carbon), and butyric (4-carbon) acids are produced by the gut microbiota and also ingested in the human diet. Intestinal SCFA uptake is a transport function of importance to humankind in that it is the principal means by which domestic cattle obtain nourishment, enabling the production of dairy products and meat for human consumption (6). In the hindgut, luminal SCFAs increase colonic contractility and the secretion of Cl−, HCO3−, and mucus via neural and nonneural pathways (33, 45, 58, 59). Moreover, SCFAs facilitate Na+ and Ca2+ absorption (51, 53). In the rat jejunum and ileum, intraluminal or jugular venous injection of SCFAs increases transmural potential difference in vivo (55, 60). Nevertheless, foregut SCFA sensing and mucosal responses to luminal SCFAs have rarely been investigated, likely due to a perceived relative lack of bacterial colonization in proximal gut segments.

The duodenum possesses specialized chemosensory functions as part of an “early warning system” alerting the distal gut and neighboring organs to proximal conditions. We have previously demonstrated that mucosal defense mechanisms, including mucosal blood flow and mucus and HCO3− secretion, are activated by numerous luminal small molecules present after meal ingestion (reviewed in Ref. 3). Not only gastric acid, but also luminal amino acids, bile acids, and fatty acids activate defense mechanisms and the release of insulinotropic and intestinotrophic gut hormones (2, 5, 24, 56). The foregut mucosa encompasses several chemosensory mechanisms, in particular nutrient receptors expressed on enteroendocrine cells. The nutrient receptors include all known G protein-coupled receptor free fatty acid (FFA) receptors. The medium- and long-chain fatty acid receptors FFA1 (GPR40) and FFA4 (GPR120) are expressed in glucagon-like peptide (GLP-1 and -2)- and gastric inhibitory peptide (GIP)-producing enteroendocrine L and K cells (15, 20, 38). FFA2 (GPR43) and FFA3 (GPR41) are SCFA receptors; in colon, FFA2 is mostly expressed in L cells (27, 50), whereas duodenal serotonin (5-HT)-containing enterochromaffin cells express FFA2 (2, 26). FFAs are expressed in GLP-1-, peptide YY-, cholecystokinin-, GIP-, and/or secretin-producing cells in the small intestine (26, 27). Activation of enteroendocrine nutrient receptors is coupled to gut hormone release, which in turn has been implicated in the generation of functional gut symptoms and also in the pathogenesis of metabolic disorders (17).

Most recently, we reported that perfusion of the duodenal lumen with the selective FFA1 agonist GW-9508 and SCFAs elicited GLP-2 release with a consequent increased rate of HCO3− secretion, whereas the FFA2 agonist phenylacetamide-1 increased the rate of HCO3− secretion via 5-HT4 and muscarinic receptor activation independent of the GLP-2 pathway (2). These results suggest that FFA2 and FFA3 activate distinct secretory pathways in duodenum. Although acetate is an agonist for FFA2 and FFA3, the potency order differs among animal species. In humans, FFA2 has a higher affinity to acetate than does FFA3, but in mice FFA2 and FFA3 are activated equally by acetate (22). Therefore, luminal acetate may stimulate 5-HT4 and GLP-2-mediated HCO3− secretion via FFA2 and FFA3. Indeed, in rat duodenum, luminal acetate increased the rate of HCO3− secretion followed by GLP-2 release and the activation of 5-HT4 and muscarinic receptors.

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Furthermore, the monocarboxylate transporter (MCT) inhibitor α-cyano-4-hydroxycinnamic acid (4-CHCA) partially inhibited the secretory response in vivo without altering GLP-2 release, indicating an MCT-dependent secretory pathway independent of L cell activation (2). These results raise a question as to whether MCTs mediate HCO\textsubscript{3}\(^{-}\) secretion as an SCFA/HCO\textsubscript{3}\(^{-}\) exchanger on the apical membrane, or whether MCTs facilitate a SCFA absorption mechanism in the duodenum.

The \(^{13}\text{C}\)-labeled acetate breath test is used clinically to measure the gastric emptying rate. Acetate is degraded to CO\textsubscript{2} and detected in the breath within 10 min of ingestion (10), indicating rapid foregut acetate absorption. Although carrier-mediated SCFA transport was predicted by kinetic measurements in human and rat small intestine (30, 43, 44), the mechanism underlying intestinal acetate absorption is still obscure. The colonic mucosa is believed to absorb SCFAs largely via simple diffusion of the nonionic form and via SCFA/HCO\textsubscript{3}\(^{-}\) exchange, based on the observations that SCFA transport is facilitated by low luminal pH and related to luminal HCO\textsubscript{3}\(^{-}\) accumulation (13, 42). Nevertheless, SCFAs are substrates of solute carrier proteins (SLCs), including the SLC16 isoforms MCT1–4 (35) and members of the SLC5 Na\textsuperscript{+}-dependent monocarboxylate transporter (SMCT) family SMCT1 (SLC5A8) and -2 (SLC5A12) (reviewed in Ref. 18). SMCT1 is electrogenic due to its 1:2 or 1:3 stoichiometry with Na\textsuperscript{+} (34). SMCT2 neutrally transports SCFA with Na\textsuperscript{+}/1:1, whereas MCTs are either H\textsuperscript{+} cotransporters or HCO\textsubscript{3}\(^{-}\) exchangers. The topography and segmental heterogeneity of transporter expression was reported in human and mouse intestine (19, 25, 49), but the precise expression pattern in the intestine has not been previously reported in any animal species. In the present study, we investigated the localization and absorptive function of SMCT1 and MCTs in rat proximal duodenum.

METHODS

**Animals.** Male Sprague-Dawley rats weighing 200–250 g (Harlan, San Diego, CA) were fed a pellet diet and water ad libitum. All studies were performed with approval of the Veterans Affairs Institutional Animal Care and Use Committee. Rats were fasted overnight with free access to water before the experiments.

**Chemicals.** UK-5099 was obtained from Tocris Bioscience (Ellisville, MO). Sodium ibuprofen, sodium acetate, sodium butyrate, sodium propionate, sodium L-lactate, 4-CHCA, and other chemicals were purchased from Sigma Chemical (St. Louis, MO). Ibuprofen, acetate, butyrate, propionate, and L-lactate were dissolved in distilled water. UK-5099, amiloride, bumetamide, ouabain, and 4-CHCA were dissolved in dimethyl sulfoxide (DMSO). Indomethacin was dissolved in 100% ethanol.

**Effect of acetate on duodenal HCO\textsubscript{3}\(^{-}\) secretion in vivo.** Duodenal loops were prepared and perfused as previously described (4). In brief, under isoflurane anesthesia (2%), a 2-cm-length proximal duodenal loop was perfused with normal saline by using a peristaltic pump at 1 ml/min. The perfusate was bubbled with 100% O\textsubscript{2} and was stirred and warmed at 37°C with a heating stirrer. The pH of the perfusate was kept constant at pH 7.0 with a pH stat (models PHM290 and ABU901; Radiometer Analytical, Lyon, France). To eliminate buffering by added compounds, which would affect the pH-stat titration volume, flow-through pH and CO\textsubscript{2} electrodes (Lazar Research Laboratories, Los Angeles, CA) were connected to the perfusion loop enabling the simultaneous and continuous measurement of perfusate pH and CO\textsubscript{2} concentrations ([CO\textsubscript{2}]). Because the input (perfusion) [CO\textsubscript{2}] is \(\approx 0\), the effluent [CO\textsubscript{2}] and pH were used to calculate the total CO\textsubscript{2} output equivalent to the secreted HCO\textsubscript{3}\(^{-}\). To prevent contamination of the perfusate from bile or pancreatic juice, the pancreatobiliary duct was ligated just proximal to its insertion in the duodenal wall and was cannulated with a PE-10 tube to drain the juice. After stabilization with continuous perfusion of pH 7.0 saline for \(\approx 30\) min, the time \((t)\) was set as \(t = 0\). The duodenal loop was perfused with pH 7.0 saline from \(t = 0\) min until \(t = 10\) min (basal period). The perfusate was then changed to pH 7.0 Krebs buffer containing acetate (0.1 mM, pH adjusted to 7.0) from \(t = 10\) min until \(t = 35\) min (experiment period). At \(t = 10\) min, the system was gently flushed so as to rapidly change the composition of the perfusate. Duodenal HCO\textsubscript{3}\(^{-}\) secretion was expressed as total CO\textsubscript{2} output ([mmol·min\(^{-1}·cm\textsuperscript{-2}\)]) calculated from the measured pH and [CO\textsubscript{2}] in the effluent solution as previously reported (4).

Some animals were deafferented with high-dose capsaicin pretreatment (125 mg/kg sc) 10–14 days before the experiments or were pretreated with indomethacin (5 mg/kg sc) to inhibit cyclooxygenase (COX) activity 1 h before the experiments as previously described (1).

**Measurement of acetate concentration in portal venous blood.** The portal vein (PV) was cannulated using a 23-gauge needle attached with a PE-50 tube filled with heparin-containing saline as described previously (56). Two hundred microliters of venous samples were collected using a syringe with 1 μL EDTA at 0.5, 10, and 30 min after luminal perfusion with acetate (10 mM) with or without the nonsubstrate SMCT1 inhibitor ibuprofen (1 mM) or the potent MCT inhibitor UK-5099 (1 μM) (11). At the end of the experiments, arterial blood was collected from the abdominal aorta as a reference. Each sample was immediately centrifuged at 5,000 g for 5 min, and plasma was stored at \(-80\)°C until measured. Acetate concentration was measured using the Acetate Colorimetric Assay Kit (BioVision, Milpitas, CA), according to the manufacturer’s protocol.

**Tissue preparation for Ussing chamber study.** Rats were anesthetized by isoflurane and killed by exsanguination. The mucosa-submucosa preparations were obtained from rat proximal duodenum, which is the segment between a point 0.5 cm distal to the pyloric ring and the insertion point of pancreatobiliary duct. The duodenal segments were opened along the mesenteric border, and the tunica muscularis was stripped with fine forceps under a stereomicroscope in ice-cold Krebs buffer containing 10 μM indomethacin. Two preparations were prepared from each segment by dividing each longitudinally, which were then mounted between two hemichambers with an aperture = 0.3 cm\(^2\) (Physiologic Instruments, San Diego, CA).

**Short-circuit current measurement in Ussing chambers.** Chambers were bathed with serosal and luminal bathing solutions in a volume of 4 ml each, maintained at 37°C using a water-recirculating heating system. The serosal bathing solution contained (in mM) 120 NaCl, 4 KCl, 1.8 CaCl\textsubscript{2}, 25 NaHCO\textsubscript{3}, 10 HEPES (pH 7.4), 10 glucose, and 0.101 indomethacin, whereas luminal bathing solution contained 136 NaCl, 2.6 KCl, 1.8 CaCl\textsubscript{2}, 10 HEPES (pH 7.4), and 10 mannitol. The serosal bath was bubbled with 95% O\textsubscript{2}/5% CO\textsubscript{2} and the luminal bath was bubbled with 100% O\textsubscript{2}. For the Na\textsuperscript{+}-free solution, NaCl was replaced with N-methyl-d-glucamine (NMDG). For the HCO\textsubscript{3}\(^{-}\)-free condition, NaHCO\textsubscript{3} was replaced with NaCl, and acetazolamide (0.2 mM) was added to both serosal and luminal baths bubbled with 100% O\textsubscript{2}. The tissues were short-circuited by a voltage clamp (Physiologic Instruments) at zero potential difference with automatic compensation for solution resistance. Short-circuit current (\(I_\text{s}\)) was continuously measured with tissue conductance (\(G_t\)) determined every 20 s. An increase of \(I_\text{s}\) indicates luminal-to-serosal current flow, e.g., anion secretion or cation absorption. The current was recorded by the DataQ system (Physiologic Instruments). The tissues were stabilized for 30 min before the effects of SCFAs and other drugs were investigated. DMSO <0.3% in the bathing solution did not affect the basal \(G_t\).

**Immunohistochemistry and real-time PCR.** Small pieces of rat intestine and submandibular glands were immersed overnight at 4°C in Zamboni’s fixative containing 2% paraformaldehyde and 0.2% picric acid. The fixed tissues were washed in PBS (pH 7.4) containing

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20% sucrose for cryoprotection and were frozen with optimal-cutting temperature compound (Sakura Finetek, Torrance, CA). Sections were cut at 8-μm thickness and stored at −20°C until use. After thawing, the sections were postfixed and permeabilized in cold acetone followed by 0.1% Triton X-100 in PBS. Nonspecific immunoreactions were blocked by preincubation with 1% BSA/PBS solution for 30 min at room temperature. Affinity-purified rabbit MCT1 and guinea pig MCT4 antibodies were raised against each COOH-terminal peptide of murine MCT sequence according to the method previously described (57). The selectivity and specificity of these antibodies were confirmed by Western blotting of human embryonic kidney (HEK293T) cells expressing murine MCT1, MCT2, or MCT4 and of the proximal duodenal mucosa from rats. MCT antibodies and the rabbit SMCT1 antiserum (25) were diluted with 1% BSA/PBS solution to a concentration of 1 g/ml and reacted with the preblocked sections overnight at 4°C. After the sections were rinsed in PBS, fluorescence-conjugated secondary antibodies were reacted for 1 h at room temperature. The sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and observed by confocal laser microscope (LSM 710; Carl Zeiss, Jena, Germany), and images were captured with a cooled charge-coupled device digital camera system (Axio Imager Z1; Carl Zeiss).

Expression of MCT1 and MCT4 in the tissues was also analyzed by real-time reverse transcription-PCR as previously described (5). The PCR primer sequences were rat MCT1: sense (5′-GCT-GCTTCTGTGTTGCGAA-3′) and antisense (5′-CGATGAT-GAGGATCACGC-CA-3′), giving rise to a 298-bp PCR product; rat MCT4: sense (5′-acggcaggtttcataacagg-3′) and antisense (5′-AAACTTTGCTGCTGCTCAGC-3′), giving rise to a 313-bp PCR product; β-actin was used as an internal control. The expression level was presented as fold induction per 10^3 copies of β-actin by the ΔCt method.

Statistics. Values are expressed as means ± SE, and n represents the number of rats. The significance of the difference between data was determined by one-way ANOVA followed by post hoc Tukey’s test. *P < 0.05 vs. Bm (n = 5). MCT1 (red, Gb) and MCT4 (red, Hb) were faintly stained in rat submandibular glands. The sections were counterstained with DAPI (blue) and phalloidin (green) for comparison (Gc and Hc). Bar = 50 μm. I and J: immunoblotting of HEK293T cells expressing murine MCT1, MCT2, or MCT4 with MCT1 (l) and MCT4 (J) antibodies. K: Western blotting of the scraped proximal duodenal mucosa for MCT1 and MCT4.

Fig. 1. Localization of Na+–coupled monocarboxylate transporter (SMCT1) and monocarboxylate transporter (MCT) in rat duodenum and midjejunum. A and B: SMCT1 immunoreactivity (green) was present in the brush border in the duodenum (A) but not in the midjejunum (B). C and D: distinct membrane expression of apical SMCT1 (green) with basolateral MCT4 (red) in the duodenum. MCT4 immunoreactivity was detected on the epithelial basolateral membrane in the upper half of villi. E and F: MCT1 immunoreactivity was detected on the epithelial basolateral membrane in villi and crypts in the duodenum. Bar = 100 (A–C and E) and 20 (D and F) μm. Counterstained with 4′,6-diamidino-2-phenylindole (DAPI, blue). G and H: negative control for MCT1 (G) and MCT4 (H) expression. mRNA expressions of MCT1 (Ga) and MCT4 (Ha) in the submandibular glands (SMG) were significantly lower than in the duodenal bulb mucosa (Bm). *P < 0.05 vs. Bm (n = 5). MCT1 (red, Gb) and MCT4 (red, Hb) were faintly stained in rat submandibular glands. The sections were counterstained with DAPI (blue) and phalloidin (green) for comparison (Gc and Hc). Bar = 50 μm. I and J: immunoblotting of HEK293T cells expressing murine MCT1, MCT2, or MCT4 with MCT1 (l) and MCT4 (J) antibodies. K: Western blotting of the scraped proximal duodenal mucosa for MCT1 and MCT4.
of the control and experimental groups was determined by one-way or two-way ANOVA. The multiple comparisons were performed by Fischer’s least-significant difference test or Dunnett’s test. Differences were considered significant when P values < 0.05.

RESULTS

Localization of SMCT and MCT proteins in rat proximal duodenum. Immunoreactivity for SMCT1 was localized to the brush border of the enterocytes in the upper two-thirds of the villi in rat proximal duodenum (Fig. 1A). Consistent with a previous report (49), SMCT1 was not expressed in the midjejunum (Fig. 1B). MCT4 was localized to the basolateral membrane of epithelial cells lining the upper half of the villi (Fig. 1, C and D), whereas MCT1 was ubiquitously detected on the basolateral membrane of the enterocytes in villi and crypts (Fig. 1, E and F). In contrast, submandibular glands were faintly stained with MCT1 (Fig. 1Gb) and MCT4 (Fig. 1Hb), consistent with low mRNA expression (Fig. 1, Ga and Ha).

Antibody selectivity for MCT1 and MCT4 was confirmed by Western blotting with HEK293T cells expressing each MCT homolog. No cross-reaction to other MCTs was observed (Fig. 1A).

Western blotting with proximal duodenal mucosa yielded a unique band for MCT1 and MCT4 (Fig. 1K). Antibody selectivity for MCT1 and MCT4 was confirmed by Western blotting with HEK293T cells expressing each MCT homolog. No cross-reaction to other MCTs was observed (Fig. 1, I and J). Western blotting with proximal duodenal mucosa yielded a unique band for MCT1 and MCT4 (Fig. 1K).

Effect of luminal acetate on Isc in rat proximal duodenum. In Ussing chambered mucosa-submucosa preparations, the cumulative addition of acetate (pH 7.4, 1–30 mM) into the luminal bath increased \( I_{sc} \) in a dose-dependent manner (Fig. 2, A and C). When 10 or 30 mM of acetate was added, \( I_{sc} \) peaked 1–3 min after addition, sustained at an elevated value for >10 min, accompanied by an increase of \( G_{sc} \) and potential difference. Subsequent addition of ouabain (0.5 mM) into the serosal bath decreased the acetate-evoked \( I_{sc} \) to basal levels (Fig. 2A). Pretreatment with ouabain (0.5 mM) gradually decreased basal \( I_{sc} \) by 51.8 ± 17.2 \( \mu A/cm^2 \) (n = 4), stabilized within 25 min. Ouabain pretreatment significantly reduced the response to luminal acetate (Fig. 2, B and C). These results suggest that acetate-induced \( I_{sc} \) response is dependent on basolateral Na\(^+\)–K\(^+\)–ATPase activity.

Effect of bumetanide on acetate-induced \( I_{sc} \) increases. The Na\(^+\)–K\(^+\)–2Cl\(^-\) cotransporter (NKCC) 1 inhibitor bumetanide was used to investigate the ionic components of the acetate-induced increase in \( I_{sc} \). Serosal application of bumetanide (0.1 mM) decreased basal \( I_{sc} \) by 27.0 ± 6.0 \( \mu A/cm^2 \) (n = 5), stabilized within 5 min, indicating that basolateral NKCC1 is involved in electrogenic Cl\(^-\) secretion, contributing to basal \( I_{sc} \) in rat proximal duodenum. The presence of bumetanide exaggerated the dose-dependent \( I_{sc} \) response to the luminal application of acetate (Fig. 3, A and B). To minimize the confounding effect of NKCC1-dependent ion transport on the acetate-induced \( I_{sc} \) response, the succeeding experiments were performed in the presence of bumetanide.

Effect of amiloride on acetate-induced \( I_{sc} \) increases. Amiloride (0.1 mM) was used to examine the involvement of

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Fig. 2. Acetate-induced increases in short-circuit current (\( I_{sc} \)) and the effect of ouabain in Ussing chambered mucosa-submucosal preparation of rat duodenum. A: a representative \( I_{sc} \) trace illustrated that cumulative addition of acetate to the luminal bathing solution (M) increased and sustained \( I_{sc} \). The further addition of the Na\(^+\)–K\(^+\)–ATPase inhibitor ouabain to the serosal bath (S) gradually decreased the acetate-induced \( I_{sc} \) to the basal level. B: a representative time course of \( \Delta I_{sc} \) illustrated that ouabain alone decreased the basal \( I_{sc} \) and suppressed the response to luminal acetate. Ouabain was added to the serosal bathing solution 20 min before acetate application. The change of \( I_{sc} \) (\( \Delta I_{sc} \)) was calculated as the difference between \( I_{sc} \) measured just before the addition of acetate (time = 0) and peak \( I_{sc} \) values. C: acetate-induced \( \Delta I_{sc} \) was significantly reduced by ouabain pretreatment; n = 4. *P < 0.05 vs. vehicle group.
epithelial Na⁺ channels in the response to luminal acetate. In the presence of bumetanide, luminal application of amiloride had no effect on basal Iₑ, indicating negligible electrogenic Na⁺ absorption in the duodenum. Five minutes after amiloride application, acetate was cumulatively added to the luminal bath. Pretreatment with amiloride did not change acetate-induced increases in Iₑ (Fig. 3C), suggesting that SCFA-related Iₑ is not due to electrogenic Na⁺ channel activity such as ENaC.

**Effect of Na⁺ or HCO₃⁻ depletion on acetate-induced Iₑ increases.** Replacement of luminal Na⁺ with NMDG gradually decreased basal Iₑ and abolished the response to luminal acetate (1–30 mM) (Fig. 3D), suggesting that acetate-induced Iₑ response is luminal Na⁺-dependent.

Removing HCO₃⁻ from the serosal solution did not affect basal Iₑ, indicating that electrogenic HCO₃⁻ transport contributes minimally to the generation of the basal Iₑ in rat proximal duodenum. The absence of serosal HCO₃⁻, however, significantly reduced acetate-induced increases in Iₑ in the presence of bumetanide (Fig. 3, E and F), suggesting that the acetate-generated Iₑ requires serosal HCO₃⁻.

**Effect of monocarboxylate transport inhibitors on acetate-induced Iₑ increases.** A nonsubstrate SMCT1 inhibitor, ibuprofen (14), was used to examine the contribution of SMCT1 to the acetate-induced Iₑ response in rat duodenum. Luminal application of ibuprofen (0.1 or 1 mM) following bumetanide had no effect on basal Iₑ. Acetate-induced Iₑ was dose-dependently reduced by the addition of ibuprofen (Fig. 4A). The absence of a direct toxic or injurious effect of ibuprofen was supported by the lack of effect of ibuprofen on the response to serosally applied carbachol (10 μM), which transiently increased Iₑ in the presence of bumetanide and acetate to the same extent with (52.0 ± 10.7 μA/cm²) or without (43.8 ± 13.5 μA/cm²) 1 mM ibuprofen (P > 0.05).

Addition of the MCT inhibitor 4-CHCA (1 mM) to the serosal bath did not change basal Iₑ, whereas 4-CHCA significantly reduced the Iₑ response to 30 mM acetate (Fig. 4B). The potent and specific MCT inhibitor UK-5099 (10 μM) added to the serosal bath significantly reduced the Iₑ response to 30 mM acetate, whereas luminal treatment had no significant effect (Fig. 4C).

**Effect of luminal SMCT1 substrates on Iₑ in rat proximal duodenum.** To confirm the functional expression of SMCT1, we investigated the effects of established SMCT1 substrates: l-lactate, propionate, and butyrate. In the presence of bumetanide, the stepwise application of these substrates (pH 7.4, 1–30 mM) increased Iₑ dose-dependently, similar to the response to acetate (Fig. 5, A and B).

**Effect of capsaicin or indomethacin on acetate-induced HCO₃⁻ secretion in vivo.** Acetate itself is absorbed in the duodenum. Five minutes after amiloride application, acetate was cumulatively added to the luminal bath. Pretreatment with amiloride did not change acetate-induced increases in Iₑ (Fig. 3C), suggesting that SCFA-related Iₑ is not due to electrogenic Na⁺ channel activity such as ENaC.

**PV acetate concentrations during duodenal perfusion with acetate.** To confirm the presence of transmucosal acetate transport, PV plasma acetate concentrations were measured before and after luminal perfusion of acetate with or without an SCFA transporter inhibitor. Acetate concentrations in the arterial blood at the end of experiments were similar to those in PV before the perfusion of acetate (data not shown). Stable acetate concentrations were observed in the PV during luminal perfusion of pH 7.0 Krebs as a control group (Fig. 7). PV acetate concentration in the fasted rats was ~600 μM, as determined with gas chromatography (23), similar to our results. Luminal perfusion of acetate (10 mM) markedly increased PV acetate concentrations at 5 min after starting the perfusion, remaining elevated for 30 min. Copерfusion of the nonsubstrate SMCT1 inhibitor ibuprofen (1 mM) or the specific MCT inhibitor UK-5099 (1 μM) with acetate abolished the increase of PV acetate concentrations, indicating that acetate absorption by the duodenal mucosa was mediated by SMCT1 and MCT.

**DISCUSSION**

We have demonstrated the functional expression of SMCT1 on the enterocyte brush border and MCTs on the basolateral membrane, which account for acetate absorption in the proximal duodenum. Furthermore, we have shown that luminal acetate stimulates duodenal HCO₃⁻ secretion in vivo partially via activation of capsaicin-sensitive afferent nerves and the COX-dependent pathways. We recently reported that luminal acetate increased duodenal HCO₃⁻ secretion through FFAs expressed on enteroendocrine cells followed by GLP-2 release and the stimulation of 5-HT-dependent neural pathways and, additionally, through an MCT-dependent pathway (2). These results indicate that luminal acetate activates multiple mucosal responses, including gut hormone release, prostaglandin (PG) production, and afferent nerve activation, mediated by endocrine FFAs and by SMCT1 and MCTs expressed in the duodenal enterocytes. These findings underscore the importance of SCFAs in duodenal chemosensing and the regulation of mucosal defenses.

In Ussing chambered duodenal mucosa, luminal acetate increased luminal Na⁺-dependent and ouabain-sensitive Iₑ, consistent with the presence of a Na⁺-dependent electronic...
SCFA transport mechanism. Immunoreactivity for SMCT1 in the duodenal brush border further supports the functional expression of SMCT1 in duodenal enterocytes. SMCT1 is a unique MCT whose electrogenicity is attributable to its asymmetric transport properties; 2–3 Na⁺:1 monocarboxylate (34). Acetate-induced $I_{sc}$ was enhanced by NKCC1 inhibition but was not affected by ENaC inhibition, indicating that acetate stimulates ENaC-independent, electrogenic Na⁺ absorption rather than anion secretion. Because NKCC1 provides intracellular Cl⁻ for electrogenic anion secretion, the decrease of basal $I_{sc}$ due to the application of the NKCC1 inhibitor bumetanide indicates that Cl⁻ secretion contributes to the measured $I_{sc}$. Na⁺ uptake from the serosal solution into the epithelial cells via NKCC1 increases intracellular Na⁺ concentration, decreasing the transmembrane Na⁺ gradient, therefore reducing the $I_{sc}$ signal attributable to SMCT1 activity. An acetate-induced $I_{sc}$ was observed at substrate concentrations $\geq 1$ mM, consistent with the $K_m$ calculated for human SMCT1 (2.46 mM) for acetate transport in an oocyte expression system (34). Although the lack of a selective inhibitor of SMCT1 impairs the study of SMCT1 function in vivo, the nonsubstrate SMCT1 inhibitor ibuprofen (1 mM) inhibited acetate-induced $I_{sc}$ increases in vitro in the presence of indomethacin, consistent

**Fig. 4.** Effect of monocarboxylate transport inhibitors on acetate-induced $I_{sc}$ increases. Luminal acetate-induced $\Delta I_{sc}$ was measured in the presence or absence of the SMCT1 inhibitor ibuprofen (Ibu, A), the MCT inhibitors α-cyano-4-hydroxycinnamic acid (4-CHCA, B), or UK-5099 (C). Ibu was added into the luminal whereas 4-CHCA was added into the serosal bathing solution. UK-5099 followed by 30 mM acetate was added to the luminal (M) or serosal (S) bath. Pretreatment with 1 mM ibuprofen, 4-CHCA, or serosal UK-5099 significantly decreased the response to luminal acetate; $n = 3–5$. *$P < 0.05$ vs. the vehicle group.

**Fig. 5.** Effect of SMCT1 substrates on $I_{sc}$. A: representative time courses illustrated luminal SMCT1 substrate-induced $\Delta I_{sc}$. SMCT1 substrates acetate, propionate, lactate, or butyrate were cumulatively added into the luminal bath. Pretreatment with 1 mM ibuprofen, 4-CHCA, or serosal UK-5099 significantly decreased the response to luminal acetate; $n = 3–5$. *$P < 0.05$ vs. the vehicle group.
with the inhibition of SCFA uptake by ibuprofen in SMCT1-expressing oocytes (14), independent of the COX inhibitory effects of ibuprofen. These findings suggest that apical SMCT1 electrogenerically cotransports luminal acetate and Na\(^+/H^+\) into duodenal epithelial cells.

SMCT1 is highly expressed in mouse distal ileum and large intestinal epithelial cells, where luminal SCFA concentration can exceed 100 mM (47). The localization of SMCT1 to the apical membrane of colonic epithelial cells in the midcrypt region (25) supports SMCT1-mediated absorption of luminal SCFAs. In perfused rat colon, acetate absorption was partially Na\(^+/H^+\)-dependent (53). Nevertheless, SMCT1-dependent SCFA absorption has not been confirmed with broken preparations such as isolated colonocytes or brush-border membrane vesicles (31, 52). Because colonic surface cells lack SMCT1 expression (25), isolated surface colonocytes or brush-border membrane vesicles may not be good model systems for the study of SMCT1 function.

MCT1 and MCT4 were expressed in the basolateral membrane of duodenal epithelial cells, consistent with MCT1 and MCT4 localization on the basolateral membrane of epithelial cells in human, mouse, and rat large intestine (19, 25). In contrast, MCT1 expression on the enterocyte apical membrane is reported in human colon (19) and substrate-exposed rat colon (8). Luminal application of the nonspecific MCT inhibitor 4-CHCA has no effect on transmucosal SCFA transport in Ussing chambered mouse cecum (29), indicating that it is unlikely that MCTs function on the apical membrane. In rat duodenum, 4-CHCA partially inhibited acetate-induced HCO\(_3^-/H^+\) secretion in vivo (2) and inhibited electrogenic acetate absorption in Ussing chamber measurements, suggesting that acetate exits the enterocyte via basolateral MCTs. Moreover, serosal
application of the potent and specific MCT inhibitor UK-5099 decreased electrogenic acetate absorption, supporting the basolateral localization of MCTs. Furthermore, PV acetate concentration was rapidly increased by duodenal perfusion with acetate in an ibuprofen- and UK-5099-inhibitable manner, indicating that rapid monocarboxylate absorption is mediated by SMCT1 and MCTs in rat duodenum.

We have also reported in vivo experiments that luminal acetate-induced HCO₃⁻ secretion was mediated by activation of capsaicin-sensitive afferent nerves. Because luminal acetate was rapidly absorbed and MCT inhibitor reduced acetate-induced HCO₃⁻ secretion, our results suggest that the absorbed acetate stimulates subepithelial afferent nerves. Capsaicin-sensitive chemosensory vagal and spinal afferent nerves, which innervate the foregut, actuate gastrointestinal functions through interaction with intrinsic secretomotor neurons and PG production (40, 41, 48, 54). The rat proximal duodenum is densely innervated by branched vagal afferent nerves, extending nearly to the enterocyte basolateral membrane (7, 39). Our preliminary study showed that FFAs were expressed in rat nodose and dorsal root ganglia, the origins of extrinsic afferent nerves (26). These findings support our hypothesis that luminal acetate is transported across the villous epithelium into the subepithelial space, where activation of FFAs expressed on capsaicin-sensitive afferent nerves may occur. Taking these observations together, the primary function of SCFAs absorbed by the duodenum may be chemosensory rather than nutritional. A schema of duodenal SCFA absorption as part of a physiological absorptive, chemosensing, and signaling system is depicted in Fig. 8.

Among SCFAs, acetic acid is frequently ingested in the diet, since the common condiment vinegar is 4–7% acetic acid by volume (~0.7–1.2 M). Oral commensal bacteria are another source of acetate; after overnight fasting, 4–6 mM acetate can be measured in human saliva and 0.4 mM in duodenal juice (9, 11001). The common condiment vinegar is 4–7% acetic acid by volume (~0.7–1.2 M). Our preliminary study showed that FFAs were expressed in rat nodose and dorsal root ganglia, the origins of extrinsic afferent nerves (26). These findings support our hypothesis that luminal acetate is transported across the villous epithelium into the subepithelial space, where activation of FFAs expressed on capsaicin-sensitive afferent nerves may occur. Taking these observations together, the primary function of SCFAs absorbed by the duodenum may be chemosensory rather than nutritional. A schema of duodenal SCFA absorption as part of a physiological absorptive, chemosensing, and signaling system is depicted in Fig. 8.

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If SCFAs are not ingested in the diet, their presence in the gut lumen is indicative of bacterial activity. Therefore, luminal SCFA sensing may be important for the maintenance of symbiosis between host and gut microbiota. Human amniotic fluid contains organic acids, including acetate and lactate at concentration 6.9 and 9.7 mM, respectively (32). In infants, feces contain significant amounts of acetate and lactate, but rarely propionate and butyrate, produced by bacterial fermentation from oligosaccharides contained in breast milk (16). These reports suggest that the human intestine is exposed to monocarboxylates throughout life and even prenatally, further supporting the key functions of duodenal SCFA sensing and absorption mechanisms. Because SCFAs stimulate hormone release from enteroendocrine cells and absorbed SCFAs activate afferent nerves, SCFA sensing and absorption mechanisms in the duodenum may also provide a logical basis for the pathogenesis of functional dyspepsia syndromes in the presence of intestinal bacterial overgrowth.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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