Acetic Acid Suppresses the Increase in Disaccharidase Activity That Occurs during Culture of Caco-2 Cells[1,2]

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ABSTRACT To understand how blood glucose level is lowered by oral administration of vinegar, we examined effects of acetic acid on glucose transport and disaccharidase activity in Caco-2 cells. Cells were cultured for 15 d in a medium containing 5 mmol/L of acetic acid. This chronic treatment did not affect cell growth or viability, and furthermore, apoptotic cell death was not observed. Glucose transport, evaluated with a nonmetabolizable substrate, 3-*O*-methyl glucose, also was not affected. However, the increase of sucrase activity observed in control cells (no acetic acid) was significantly suppressed by acetic acid ($P < 0.01$). Acetic acid suppressed sucrase activity in concentration- and time-dependent manners. Similar treatments (5 mmol/L and 15 d) with other organic acids such as citric, succinic, L-maric, L-lactic, L-tartaric and itaconic acids, did not suppress the increase in sucrase activity. Acetic acid treatment (5 mmol/L and 15 d) significantly decreased the activities of disaccharidases (sucrase, maltase, trehalase and lactase) and angiotensin-I-converting enzyme, whereas the activities of other hydrolases (alkaline phosphatase, aminopeptidase-*N,* dipeptidylpeptidase-IV and γ-glutamyltranspeptidase) were not affected. To understand mechanisms underlying the suppression of disaccharidase activity by acetic acid, Northern and Western analyses of the sucrase-isomaltase complex were performed. Acetic acid did not affect the de novo synthesis of this complex at either the transcriptional or translational levels. The antihyperglycemic effect of acetic acid may be partially due to the suppression of disaccharidase activity. This suppression seems to occur during the post-translational processing. J. Nutr. 130: 507–513, 2000.

KEY WORDS: • *Caco-2 cells* • *acetic acid* • *disaccharidase*

Vinegar is used as a seasoning when food is cooked and eaten. Acetic acid is one of the main components of vinegar. The concentration of acetic acid in vinegar commercially available ranges from 4 to 15% (Coppini et al. 1975, Itoh 1978, White 1971). Dishes in which sourness is required, such as sushi, pickles and marinated food, contain 10–150 mmol/L of acetic acid (Bell et al. 1972, Maruyama et al. 1980, Rodger et al. 1984, Seuss and Martin 1993). When carbohydrates that are indigestible in the small intestine, such as dietary fiber and resistance starch, are orally ingested, they are broken down by fermentation in the large intestine, short-chain fatty acids (SCFA)[6] such as acetic, propionic and butyric acids are produced, and they are then absorbed from the large intestine (Cummings 1983). Pomare et al. (1985) measured acetic acid appearing in the venous blood after lactulose (a fermentable carbohydrate) and acetic acid were orally ingested. The blood level of acetic acid reached a peak much faster when acetic acid was ingested than when lactulose was given. Because the absorption of acetic acid from the stomach is poor, the absorption site of orally ingested acetic acid seems to be the small intestine (Meyer-Wyss et al. 1991). Furthermore, Pomare et al. (1985) have shown that the acetic acid level in the blood was similar in either case when lactulose or acetic acid was orally administered. When meals containing fermentable carbohydrate were orally administered to experimental animals, the concentration of acetic acid in cecal digesta reached ~100 mmol/L (Cheng et al. 1987, Topping et al. 1985). These studies indicate that when food containing 10–150 mmol/L of acetic acid is eaten, the concentration of acetic acid possibly reaches the millimolar level in the small intestine. Such a high concentration of acetic acid may disturb physiological func-

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[2] Ogawa, N., Satsu, H., Watanabe, H., Miyamoto, Y., Fukaya, M., Tsukamoto, Y. & Shimizu, M. (1998) Chronic administration of acetic acid decreases the activity of glycosidases in Caco-2 intestinal epithelial cells. JAACT/ESACT '98 Meeting Program & Abstracts, pp. 73.

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[6] Abbreviations used: ACE, angiotensin-I converting enzyme; ALP, alkaline-phosphatase; AP-N, aminopeptidase-*N;* DPP-IV, dipeptidylpeptidase-IV; ECL, enhanced chemiluminescence; 3-*O*-met glucose, 3-*O*-[methyl-^{3}H]-D-glucose; γ-GTP, γ-glutamyltranspeptidase; LDH, lactate dehydrogenase; NP-40, Nonidet P-40; rER, rough-surfaced endoplasmic reticulum; RT-PCR, reverse transcription-polymerase chain reaction; SCFA, short-chain fatty acids; SGLT1, sodium-dependent glucose transporter 1; S-I complex, sucrase-isomaltase complex; TEER, transepithelial electrical resistance; tGA, trans-Golgi apparatus.

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tions of the intestine. However, only a few studies concerning acetic acid have been carried out in the small intestine.

Previous in vivo experiments have shown an antihyperglycemic effect of vinegar. For example, rats were fed for ~30 d a high-sucrose diet containing 15.4% vinegar (Yonemoto et al. 1995). Blood glucose concentration was significantly lower in rats administered acetic acid. In another study, rats were given diet containing 7% vinegar for 10 wk and they were then orally loaded 250 mg of glucose per 100 g body weight. The postprandial blood glucose was significantly lower in rats fed vinegar (Ebihara and Nakajima 1988). Carbohydrates are digested by amylase which is present in saliva and pancreatic juice. Disaccharides are further hydrolyzed by glycosidases in the brush border membrane of the intestinal epithelium. Subsequently, resultant monosaccharides are absorbed from the intestine via specific sugar transport systems. To our knowledge, acetic acid at the millimolar level does not inhibit amylase. If acetic acid inhibits transport systems specific to glucose or disaccharidases in the intestine, glucose absorption from the intestine decreases, resulting in lowering the blood glucose level. This hypothesis may partially account for the antihyperglycemic effect of vinegar. Therefore, in this study we investigated effects of acetic acid on intestinal glucose transporters and disaccharidases.

To examine if acetic acid affects glucose transport or disaccharidases, we designed in vitro experiments, using a human intestinal cell line, Caco-2. This cell line is derived from a colon carcinoma although it behaves like a small intestinal epithelial cell after differentiation and polarization (Pinto et al. 1983). Therefore, this cell line has been used to study physiological functions of the small intestine (Blais et al. 1987). Caco-2 cells express glucose transporters as well as disaccharidases (Howell et al. 1992, Stein et al. 1996, Zweibaum 1991). Furthermore, post-translational processing of hydrolases, such as alkalinephosphatase (ALP), aminopeptidase-N (AP-N), dipeptidylpeptidase-IV (DPP-IV), γ-glutamyltranspeptidase (γ-GTP), angiotensin-I converting enzyme (ACE) and disaccharidases (sucrase, maltase, trehalase and lactase) has been studied in this cell line (Danielsen 1992, Garcia et al. 1993, Gilbert et al. 1991, Hauri et al. 1985, Le Bivic et al. 1990, Naim 1993 and Sjötrö et al. 1983, Stieger et al. 1988). Therefore, the Caco-2 cell line was used in the present study.

MATERIALS AND METHODS

Cell culture. A human colonic carcinoma cell line, Caco-2, was obtained from American Type Culture Collection (Rockville, MD). Caco-2 cells were routinely cultured at 37°C in a humidified atmosphere of 95% air-5% CO_2. The composition of the medium was Dulbecco's modified Eagle's medium (Nissui Pharmaceuticals, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY), 1% nonessential amino acids (Mediatech, Herndon, VA), 4 mmol/L of glutamine and 1×10^5 U/L of penicillin-100 mg/L of streptomycin (Gibco). The pH of the medium was adjusted to 7.4 with sodium bicarbonate. Fresh medium was given every 2 d, and cells were passaged before confluence at a split ratio of 1:2 by their exposure to 0.1% trypsin (Difco Laboratories, Detroit, MI) and 0.02% EDTA in PBS. Cells used were at passages 64–78.

For measurements of enzyme activity, cell growth and cell viability, cells were seeded at a density of 0.14 million cells/well in 24-well plastic microplates (Iwaki Glass, Chiba, Japan). The microplates were precoated with Type-I collagen (Nitta Gelatin, Osaka, Japan). Cells for glucose transport assays were seeded on polycarbonate membranes (Corning Costar, Cambridge, MA). In both cultures for specific experiments, cells were maintained for 1 d using the standard medium after seeding to promote their recovery from damage caused by trypsinization. The medium was then switched to that containing acetic acid or other organic acids. When we cultured cells on polycarbonate membranes, media containing acetic acid or other chemicals were applied to the apical side. The standard medium was always used on the basal side. Because the pH of the medium decreased only by 0.2 unit even when the concentration of acetic acid in the medium was 5 mmol/L (the maximal concentration of acetic acid that we used), the pH was not readjusted. For control experiments, cells were concurrently cultured with the standard medium. Under these culture conditions, Caco-2 cells seeded on the permeable membrane formed a monolayer of cells interconnected with tight junctions by d 15.

Assay of cell proliferation and viability. Cell proliferation was monitored by serial assays of cellular protein. The protein was determined by Bradford method (1976). The viability of cells was assessed by their ability to reduce alamarBlue (Biosource International, Camarillo, CA) and to maintain lactate dehydrogenase (LDH) inside cells using LDH-cytotoxic test kit (Wako, Osaka, Japan). The cell viability was also estimated by cell counting with trypan blue.

Measurement of glucose uptake. When transport measurements were carried out, medium was removed from the apical and basal sides of cell monolayers cultured on the permeable membrane. The PBS was used to rinse both sides of the monolayer as well as to preincubated at 37°C for 15 min. After PBS was removed, 600 μL of PBS was applied to the basal side and then 300 μL of PBS containing 123 nmol/L of 3-*O*-[methyl-^{3}H]-D-glucose (3-*O*-met glucose) (Amersham, Buckinghamshire, United Kingdom) was placed to the apical-side to initiate transport. The 3-*O*-Met glucose uptake into cells and influx from the apical to the basal side through the cell monolayer were estimated from the radioactivity appearing in the cell lysate and the basal solution, respectively. The uptake was measured for 5 min. To terminate uptake, radioactive glucose was removed from the apical side, and cell monolayers were washed three times with 700 μL of ice-cold PBS containing 0.5 g/L of sodium azide. Subsequently, cells were lysed with 250 μL of 0.1% Triton X-100 and transferred to counting vials. For measurements of 3-*O*-met glucose influx, 600 μL of PBS were collected from the basal side and transferred to counting vials. The radioactivity was determined by liquid scintillation spectrometry.

Determination of enzyme activity. The activities of glycosidases, sucrase, maltase, trehalase and lactase were determined by Dahlqvist's method (Dahlqvist 1964). In brief, cell monolayers were rinsed with 700 μL of PBS, and 400 μL of PBS containing 28 mmol/L of the substrate was placed on cultured monolayers. Substrates used for sucrase, maltase, trehalase and lactase assays were sucrose, maltose, D-(+)-trehalose and D-(+) lactose, respectively. After cells were incubated with a solution containing the substrate for a predetermined period at 37°C, the substrate solution was collected. The amount of glucose released from the substrates was determined using a kit (Glucose Test #273-13901; Wako). The activity of ALP was determined by liberation of *p*-nitrophenol from *p*-nitrophenylphosphate (Forstner et al. 1968). The activity of γ-GTP was assessed by measuring nitroanilide released from 3 mmol/L of L-glutamic γ-para-nitroanilide (Louvard et al. 1975). Glycylglycine was used as the recipient of γ-glutamine. The activities of AP-N and DPP-IV were determined using ala-7-amino-4-methylcoumarin and gly-pro-7-amino-4-methylcoumarin as substrates, respectively. Released methylcoumarin was assayed by spectrofluorometry (excitation; 370 nm, emission; 442 nm). The activity of ACE was determined using benzoyl-gly-his-leu as a substrate by a method described previously (Stewart et al. 1981). Hydrolyzed benzoyl-gly (hippuric acid) was spectrophotometrically assayed at 228 nm. The activities of hydrolases are expressed as μmol substrate hydrolyzed/(mg cellular protein · h).

Northern blot analysis. Cells were cultured in 100-mm dishes and treated with 5 mmol/L of acetic acid for 15 d. Total RNA was extracted from cells using Isogen RNA isolation kit (Wako). Total RNA (50 μg) was fractionated by electrophoresis on a denatured gel and transferred to a nylon membrane (Amersham). After prehybridization, the membrane was incubated with a ^{32}P-labeled probe for sucrase-isomaltase (S-I) complex obtained by reverse transcription-polymerase chain reaction (RT-PCR) from Caco-2 cells. Primers for PCR were designed from the cDNA sequence of the human S-I complex (Chantret et al. 1992): sense, 5′-TAGGTCGACGTGCCAATGGA-3′ (nucleotides 1300–1320); antisense, 5′-GCAGGATCCTGATGTTCATATC-3′ (nucleotides 2002–2023). The

membrane hybridized with the probe was washed, exposed on an image plate and analyzed with a BAS-2000 II image analyzer (Fuji Film, Tokyo, Japan). Control hybridization was carried out with human β-actin cDNA.

Western blot analysis. Caco-2 cells cultured in 100-mm dishes (6 million cells per dish) in the presence of 5 mmol/L of acetic acid for 15 d were lysed in 500 μL of a buffer whose composition was 150 mmol/L of NaCl, 1 mmol/L of EDTA and 1% of Nonidet P-40 (NP-40), buffered with 10 mmol/L of Tris/HCl, pH 8.0. The S-I complex was immunoprecipitated with 5 μL of a monoclonal antibody specific to this complex which was generously provided by Dr. H.-P. Hauri, University of Basel (Switzerland). The precipitant was separated by SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane. The location of S-I complex was detected by an ECL method using a kit (Amersham).

Statistical analysis. Each result is expressed as the means ± SEM for four or seven independent determinations. The number of determinations indicates that of experiments with separate cell monolayers which were performed at each time point or concentration. Differences between experimental and control data were assessed by Student's *t* test (Microsoft Excel, Microsoft Corporation, Roselle, IL). The *P* value (two-tailed) of <0.01 indicates significant difference.

RESULTS

To examine effects of acetic acid on the growth of Caco-2 cells, cellular protein was monitored while cells were cultured with 5 mmol/L of acetic acid for 15 d. The increase in protein of cells treated with acetic acid did not differ from that of control cells (**Fig. 1**). The effect of acetic acid on the viability of Caco-2 cells was also studied. The viability of cells was estimated from their reducing activity of alamarBlue, retention ability of LDH and excretion ability of trypan blue. Acetic acid also did not reduce the viability of Caco-2 cells (**Table 1**).

To study effects of chronic treatment of Caco-2 cells with acetic acid on glucose transport, cells seeded on permeable membranes were cultured for 15 d with or without 5 mmol/L of acetic acid. To assess the activity of glucose transport in Caco-2 cells, 3-*O*-met glucose uptake into the cells and influx

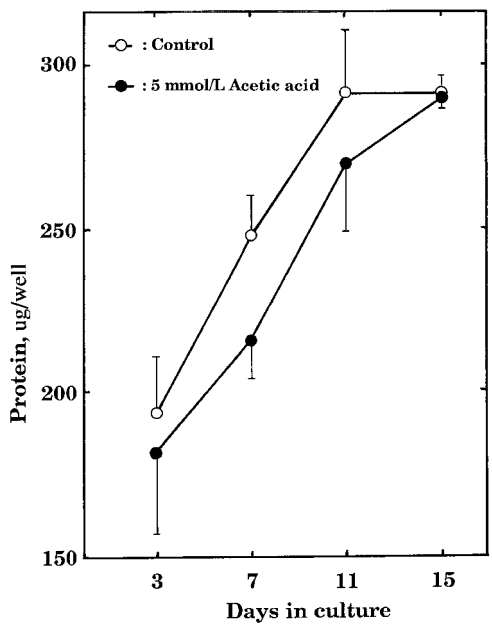

FIGURE 1 Effects of chronic treatment of Caco-2 cells with acetic acid on their growth. Caco-2 human intestinal epithelial cells were cultured for 15 d with or without 5 mmol/L of acetic acid. Cellular protein was determined on d 3, 7, 11 and 15. The data are given as means with bars representing SEM, $n = 4$.

TABLE 1

Effect of acetic acid on the viability of Caco-2 cells[1,2,3]

	Control	Acetic acid
	%	
Reducing ability of alamarBlue	100 ± 14	100 ± 16
LDH released	3.6 ± 0.3	2.7 ± 0.4
Living cells	95 ± 0.8	96 ± 1.4

[1] Cells were treated with 5 mmol/L of acetic acid for 15 d.
[2] lactate dehydrogenase, LDH.
[3] Values are means ± SEM, $n = 4$.

from the apical to the basal side through cell monolayer were measured. Uptake or influx of 3-*O*-met glucose was not affected by the chronic treatment with acetic acid (**Fig. 2***A*, *B*).

We also studied acute effects of acetic acid on glucose transport in Caco-2 cells. Cells were not treated with acetic acid during culture. The concentration of acetic acid was varied from 0.5 to 2.5 mmol/L in the PBS applied to the apical side of cells during the transport assay. Acute exposure to acetic acid did not significantly increase 3-*O*-met glucose uptake ($P = 0.09–0.44$). (**Fig. 3***A*). The influx of 3-*O*-met glucose was not affected by the presence of acetic acid during the influx assay (Fig. 3*B*).

On d 3 of culture, sucrase activity was not significantly different in cells cultured with and without 5 mmol/L of acetic acid (**Fig. 4**). Thereafter, the sucrase activity greatly increased in control cells while that activity in cells cultured with acetic acid stayed low. The activity of sucrase in cells treated with acetic acid was significantly less than that in control cells on d 7, 11 and 15 ($P < 0.01$). This decrease in sucrase activity due to acetic acid was not due to its direct inhibition of sucrase during the assay (0 mmol/L of acetic acid in the assay mixture, 10.4 ± 0.5; 5 mmol/L of acetic acid, 11.3 ± 0.2 of μmol · mg protein^{-1} · h^{-1}; $n = 4$).

Cells were cultured for 15 d in a medium containing acetic acid in the range of 0 to 5 mmol/L. Acetic acid inhibited sucrase activity in a dose-dependent manner (**Fig. 5**). Even 1

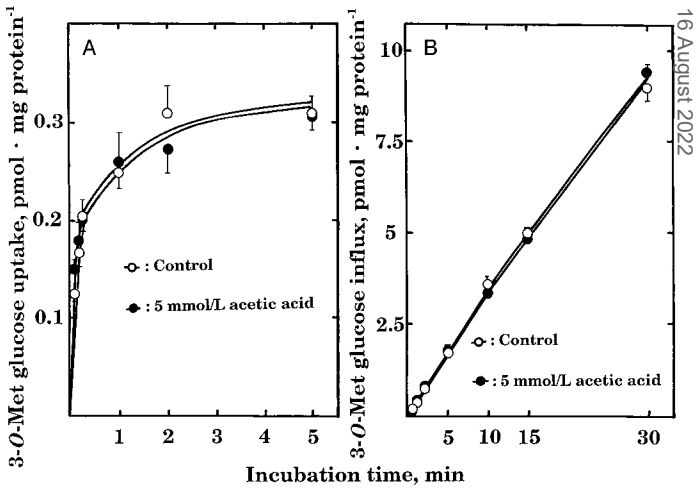

FIGURE 2 Effects of chronic treatment with acetic acid on 3-*O*-met glucose transport in Caco-2 cells. Cells were cultured for 15 d with or without 5 mmol/L of acetic acid. The uptake (panel A) and influx (panel B) of 3-*O*-met glucose were measured. The data are given as means with bars representing SEM, $n = 4$.

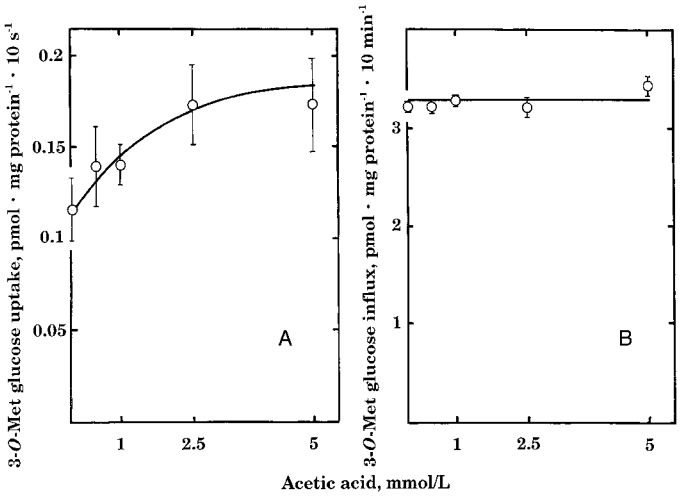

FIGURE 3 Effects of acute addition of acetic acid on 3-*O*-met glucose transport in Caco-2 cells. The concentration of acetic acid was varied from 1 to 5 mmol/L during uptake (panel A) and influx (panel B) assays. The data are given as means with bars representing SEM, $n = 4$.

mmol/L of acetic acid inhibited sucrase activity by 30% ($P < 0.01$).

Effects of chronic exposure (15 d) to various organic acids (5 mmol/L) on sucrase activity in Caco-2 cells were studied. Acetic acid decreased sucrase activity to ~50% of the control while other organic acids including citric, succinic, L-maric, L-lactic, L-tartaric and itaconic acids, did not (**Table 2**).

We studied the chronic effects of acetic acid treatment on the activities of disaccharidases and other hydrolases located in the apical membrane of Caco-2 intestinal epithelium. The activities of sucrase and maltase were suppressed to ~40% of the control after 15-d treatment with 5 mmol/L of acetic acid (**Table 3**). The activities of trehalase and lactase were almost

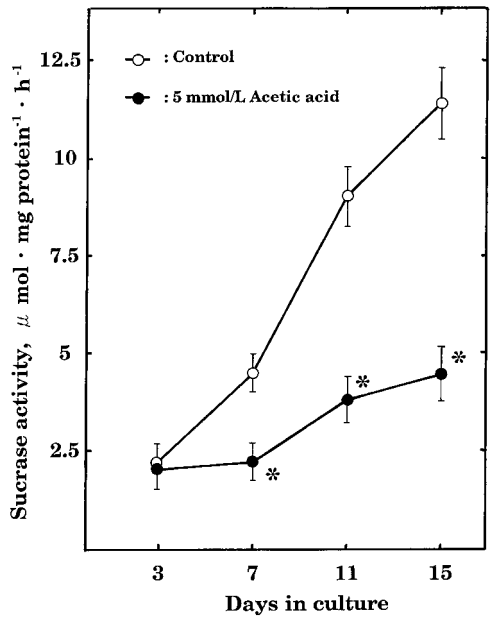

FIGURE 4 Profile of sucrase activity during culture of Caco-2 cells. Sucrase activity was monitored for 15 d during culture of Caco-2 cells with or without 5 mmol/L of acetic acid. The data are given as means with bars representing SEM, $n = 7$. *Significantly different from the control, $P < 0.01$.

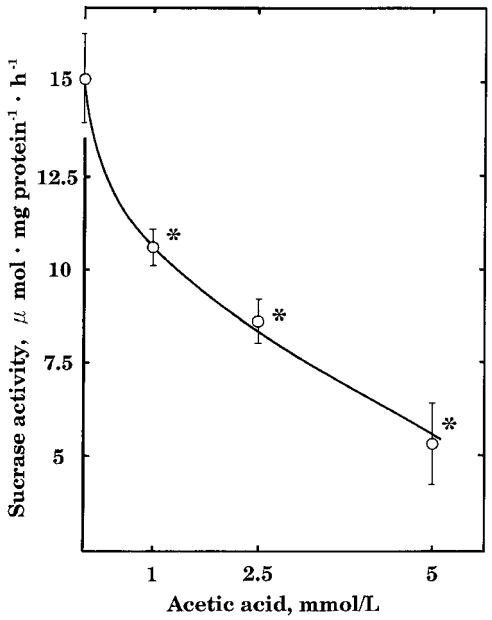

FIGURE 5 Dose-dependent suppression of sucrase activity of Caco-2 cells by acetic acid. Cells were cultured with acetic acid for 15 d. The concentrations of acetic acid were 0, 1, 2.5 and 5 mmol/L in the medium. The data are given as means with bars representing SEM, $n = 7$. *Significantly different from the control (0 mmol/L of acetic acid), $P < 0.01$.

completely inhibited. The activity of ACE was reduced to 30% of the control by acetic acid treatment while the activities of ALP, AP-N, DPP-IV and γ-GTP were not affected.

Figure 6*A* shows a representative result of Northern blot analysis using a probe for the S-I complex made by RT-PCR. A band was found at the size of 6.8 kb as previously reported (Chantret et al. 1992). Acetic acid treatment did not affect the density ratio of the S-I complex to β-actin (control, 0.87 ± 0.04; acetic acid, 0.99 ± 0.05; $P = 0.12$, $n = 3$). We performed a Western blot to examine the change in the amount of S-I complex due to acetic acid treatment. A monoclonal antibody specific to the human S-I complex was used to precipitate and detect the complex. The complex was found at 210 kDa (Fig. 6*B*). The treatment of cells with acetic acid did not change the degree of density of the band at 210 kDa (the density ratio of acetic acid to control treatments, 1.07 ± 0.14, $n = 3$).

TABLE 2

Effects of various acids on the activity of sucrase in Caco-2 cells[1,2]

Organic acids	Sucrase activity, *μmol · mg protein⁻¹ · h⁻¹*
Control	10.2 ± 0.7
Acetic acid	5.0 ± 0.2*
Citric acid	11.3 ± 0.2
Succinic acid	9.2 ± 0.3
L-Maric acid	8.1 ± 0.4
L-Lactic acid	11.4 ± 0.9
L-Tartaric acid	11.5 ± 0.4
Itaconic acid	10.4 ± 0.3

[1] Cells were treated with 5 mmol/L of various acids for 15 d.
[2] Values are means ± SEM, $n = 4$. *Significantly different from control, $P < 0.01$.

TABLE 3

Effects of acetic acid on the activity of hydrolases expressed in Caco-2 cells[1,2,3]

Hydrolases	Enzyme activity		
	Control	Acetic acid	% of control
	μmol · mg protein^{-1} · h^{-1}		
ALP	0.45 ± 0.07	0.51 ± 0.04	112
AP-N	30.2 ± 2.7	31.5 ± 3.3	104
DPP-IV	73.7 ± 3.3	69.5 ± 4.5	94
γ-GTP	1.21 ± 0.21	1.22 ± 0.24	101
ACE	0.13 ± 0.02	0.04 ± 0.01*	30
Sucrase	10.6 ± 1.5	4.6 ± 0.7*	43
Maltase	57.9 ± 4.6	22.5 ± 3.5*	39
Trehalase	1.39 ± 0.36	0.05 ± 0.07*	4
Lactase	4.42 ± 0.39	0.37 ± 0.75*	8

[1] Cells were treated with 5 mmol/L of acetic acid for 15 d.
[2] alkalinephosphatase, ALP; aminopeptidase-N, AΠ-N; dipeptidylpeptidase-IV, DPP-IV; γ-glutamyltranspeptidase, γ-GTP; angiotensin-I converting enzyme, ACE
[3] Values are means ± SEM, $n = 4$. *Significantly different from control, $P < 0.01$.

DISCUSSION

We cultured Caco-2 cells in the medium containing 5 mmol/L of acetic acid for 15 d. Cell growth was monitored during cell culture, and cell viability was examined at the end of the culture. Acetic acid did not either disturb cell growth or cell viability (Fig. 1 and Table 1), showing that 5 mmol/L of acetic acid is not harmful to the intestinal cells. These results are consistent with those in previous studies of intestinal cell lines. Gamet et al. (1992) have shown using HT-29 cells that 30 mmol/L of sodium acetate did not affect the growth of cells whereas propionate and butyrate at the same concentration were inhibitory. Even though propionate and butyrate at 4 mmol/L induced apoptosis in several colorectal tumor cell lines including Caco-2 and HT-29, the concentration of acetate required to induce apoptosis was more than 40 mmol/L (Hague et al. 1995, Marchetti et al. 1997). Under our microscopic observation, 5 mmol/L of acetic acid did not induce apoptotic cell death in Caco-2 cells, either. The mechanisms underlying apoptosis induced by propionate and butyrate in HT-29 and Caco-2 cells are not clear, although deoxycholate induces apoptosis in these cells by increasing the intracellular concentration of Ca^{2+}. Therefore, the reason why among SCFA acetic acid seems less harmful to intestinal epithelial cells is still unknown.

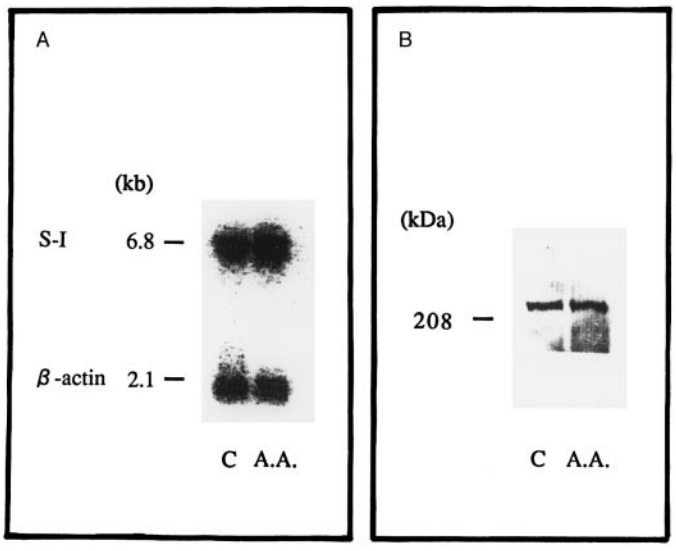

FIGURE 6 Northern and Western analyses of the complex, sucrase-isomaltase complex (S-I) complex in CaCo-2 cells cultured with 5 mmol/L of acetic acid for 15 d. Total RNA extracted from Caco-2 cells was used for a Northern analysis (*panel A*). A radiolabeled probe specific the human S-I complex was made by reverse transcription-polymerase chain reaction (RT-PCR) as described in the Materials and Methods section. The human β-actin was used as an internal control. Cell lysate made after 15-d treatment with acetic acid was immunoprecipitated with a monoclonal antibody and fractionated by SDS-PAGE. The S-I complex was visualized by an enhanced chemiluminescence (ECL) method (*panel B*). C, control; A.A., acetic acid.

To determine if acetic acid treatment inhibits glucose transport, glucose uptake into Caco-2 cells and influx through cell monolayers were measured using radiolabeled 3-*O*-met glucose as a nonmetabolizable substrate. Cells cultured for 15 d in a medium were studied. The transepithelial electrical resistance (TEER) of monolayers treated with acetic acid for 15 d was significantly higher than that of control (control; 715 ± 57Ω × cm^2, acetic acid; 1060 ± 16 Ω × cm^2, $n = 4$, $P < 0.01$). The TEER value is an index of the tightness of the intestinal monolayer barrier. Because the paracellular permeability of substances decreases as the TEER increases, we assumed less influx of glucose through the monolayer of Caco-2 cells treated with acetic acid, leading to the decrease in the glucose concentration. However, the chronic acetic acid treatment did not change the transepithelial influx of 3-*O*-met glucose (Fig. 1B). The coexistence of acetic acid during flux measurements also did not inhibit 3-*O*-met glucose influx (Fig. 2B). We also studied 3-*O*-met glucose uptake into the cells. The 3-*O*-Met glucose uptake was not different from controls in cells treated with acetic acid (Fig. 1A). The coexistence of acetic acid did not stimulate 3-*O*-met glucose uptake (Fig. 2A). However, we did not obtain data showing decreased 3-*O*-met glucose influx or uptake due to chronic or acute treatment of acetic acid. Acetic acid does not inactivate glucose transport in the intestinal cells, which does not explain the antihyperglycemic effect of acetic acid observed in vivo.

The present study has shown for the first time an inhibitory

effect of acetic acid on the increase of disaccharidase activity during the growth of Caco-2 intestinal cells (Fig. 4). The disaccharidases inhibited by acetic acid were sucrase, maltase, trehalase and lactase (Table 3). Sucrase and isomaltase are synthesized as a complex (Traber 1994). The activity of sucrase increases in the intestine of rats fed a sucrose-rich diet. This upregulation of sucrase is due to an increase in the S-I complex mRNA (Broyart et al. 1990). Simultaneously, the mRNA level of sodium-dependent glucose transporter 1 (SGLT1) is also elevated by a sucrose-rich diet (Miyamoto et al. 1993). Since sucrose is hydrolyzed to glucose by sucrase and this glucose is transported by SGLT1, this is a response of the intestine to enhance the bioavailability of sucrose. These previous studies indicate that disaccharidases are susceptible to transcriptional regulation. In this study, we performed a Northern blot analysis to examine the effect of chronic acetic acid treatment on the S-I complex mRNA level. Acetic acid did not affect the S-I complex mRNA, indicating that acetic acid is not involved in the transcriptional regulation of disaccharidases (Fig. 6A). Furthermore, a Western analysis was performed, showing that the translation of the S-I complex also is not negatively regulated by acetic acid (Fig. 6B).

A possible explanation for the suppression of disaccharidases caused by acetic acid is that acetic acid may inhibit such a post-translational processing step such as intracellular trafficing of de novo synthesized disaccharidases, their glycosylation or their sorting into the polarized membrane. Sucrase, maltase, lactase, AP-N and DPP-IV are classified as transmembrane proteins while ALP and trehalase are classified as glycosyl-phosphatidylinositol-anchored proteins (Danielsen 1992, Garcia et al. 1993). ALP and sucrase are delivered directly to the apical membrane while AP-N and DPP-IV are delivered via the basolateral membrane (Gilbert et al. 1991, Le Bivic et al. 1990). In our study, disaccharidases were suppressed by acetic acid, whereas ALP, AP-N, DPP-IV and γ-GTP were not. Therefore, the suppression of disaccharidases by acetic acid is not explained by these classifications with regard to membrane sorting or binding of the hydrolases.

Accumulated data have shown that the transport of peptidases such as AP-N and DPP-IV from rough-surfaced endoplasmic reticulum (rER) to *trans*-Golgi apparatus (*t*GA) in Caco-2 cells was considerably faster than that of disaccharidases (Hauri et al. 1985, Stieger et al. 1988). This phenomenon is called the asynchronous transport of intestinal brush-border hydrolases. ACE is also transported from rER to tGA by a slower trafficking pathway and its transport kinetics are comparable to those of disaccharidases (Naim 1993). Disaccharidases and ACE do not form homodimers, whereas AP-N and DPP-IV do (Sjötrö et al. 1983), suggesting the hypothesis that brush-border hydrolases utilize different routes from rER to tGA, according to differences in their structural maturation. A paper by Quaroni et al. (1993) supports this hypothesis because they observed that culturing Caco-2 cells at 42.5°C decreased cell surface delivery of the S-I complex but did not affect those of DPP-IV or AP-N. A Western analysis in our experiments showed that acetic acid does not decrease the amount of the S-I complex (Fig. 6), indicating that acetic acid does not affect the de novo synthesis of the S-I complex until its translation. We studied the effect of acetic acid treatment (5 mmol/L and 15 d) on the activity of brush-border hydrolases. The activities of disaccharidases and ACE were suppressed by this treatment, whereas those of ALP, AP-N, DPP-IV and γ-GTP were not (Table 2). These data are well accounted for by the hypothesis, indicating that acetic acid may interrupt a slow transport pathway from rER to tGA which is shared by disaccharidases and ACE.

In conclusion, culturing Caco-2 cells with acetic acid suppresses the increases in disaccharidase activities during cell growth. This suppression by acetic acid may be due to the interference of post-translational trafficking of synthesized disaccharidases from rER to tGA. This seems to be one of the mechanisms underlying the antihyperglycemic effect of vinegar observed in vivo. However, there are still cases which cannot be explained by our conclusion obtained in this study. Nakajima and Ebihara (1988) have shown that long-term treatment of rats with acetic acid reduces blood glucose concentration immediately after the administration of glucose. This study demonstrates that acetic acid can reduce the glucose level in the blood even when glucose itself is loaded. Brighenti et al. (1995) have shown another case in which acetic acid decreases the blood glucose level even when acetic acid is given with meal, indicating an acute effect of acetic acid on the blood glucose level. Acetic acid may change glucose tissue distribution or its utilization to synthesize glycogen. To understand mechanisms underlying the antihyperglycemic effects of acetic acid, further experiments are required.

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