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FATTY ACID-INDUCED ALTERATIONS IN TRANSPORT SYSTEMS OF THE SMALL INTESTINAL BRUSH-BORDER MEMBRANE

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Abstract—We have investigated the effects of fatty acids on the Na^+-H^+ exchanger and other carrier-mediated transport systems in intestinal brush-border membrane vesicles. The Na^+-H^+ exchanger (i.e. H^+ gradient-dependent, dimethylamiloride-sensitive Na^+ uptake) was strongly inhibited by fatty acids and the inhibition was concentration dependent. Unsaturated fatty acids showed more inhibition than saturated fatty acids. Among unsaturated fatty acids, ricinoleic acid was found to be the most potent inhibitor. Inhibition of the Na^+-H^+ exchanger by oleic acid was partially reversible, and the nature of the inhibition was found to be non-competitive with respect to Na^+. The dimethylamiloride-sensitive Na^+ uptake measured in the absence of an H^+ gradient was also inhibited by oleic acid, suggesting that the inhibition of the Na^+-H^+ exchanger by fatty acids was not due to the accelerated dissipation of the H^+ gradient. Treatment of the membrane vesicles with oleic acid also inhibited other carrier-mediated transport systems as well, such as the H^+ gradient-driven transport of glycylsarcosine and the Na^+ gradient-driven transport of D-glucose and L-alanine, whereas it did not affect the permeability of L-glucose, a non-carrier-mediated process. However, the inhibitory effects of oleic acid on the transport of D-glucose and L-alanine appeared to be related to the enhanced collapse of the Na^+ gradient rather than a direct effect on the carrier systems because transport of these solutes when measured in the absence of a Na^+ gradient ($[Na^+]_i = [Na^+]_o$) was not affected by oleic acid. These data demonstrate that fatty acids bring about significant alterations in the activities of various transport systems of the small intestinal brush-border membrane, either by directly interacting with the transport protein or by abolishing the energy source that is necessary for the transport process.

Diarrhea is often associated with steatorrhea, but the relationship between the intestinal malabsorption of fat and the appearance of more-than-normal quantities of water in feces has not been fully understood [1]. Several hypotheses have been proposed for the fatty acid-induced diarrhea which include stimulation of adenylate cyclase [2], mucosal injury [3], inhibition of enterocyte and colonocyte (Na^+–K^+)ATPase [4], altered colonic motility [5] and activation of enteric nerves [6]. Since malabsorption of various organic solutes and inorganic ions is seen in steatorrhea, it is possible that the presence of excess fatty acids in the intestinal lumen directly interferes with transport systems located in the intestinal brush-border membrane. A number of intestinal perfusion studies with fatty acids support this idea [7–10]. The purpose of this investigation was a study *in vitro* of the effects of free fatty acids on transport systems available for organic solutes and inorganic ions in brush-border membrane vesicles isolated from rabbit small intestine.

Among the various transport systems of the intestinal brush-border membrane, the Na^+–H^+ exchanger has been directly implicated in one particular type of congenital secretory diarrhea [11]. This exchanger has received considerable attention in recent years because it is present in a wide variety of animal cells and it plays a vital role in the regulation of intracellular pH, cell volume, transport of Na^+, HCO_3^- and Cl^-, and the initiation of cell growth and proliferation [12, 13]. In enterocytes and colonocytes, the Na^+–H^+ exchanger activity is enriched in the brush-border membrane [14–17]. The Na^+–H^+ exchanger in the brush-border membrane of small intestinal cells is responsible for the maintenance of a proton gradient (acid microclimate pH) at the mucosal surface [18, 19], which is the driving force for active absorption of folate [20] and small peptides [21–23]. Because the Na^+–H^+ exchanger is involved, either directly or indirectly, in the absorption of many inorganic ions and organic solutes, emphasis is given in the present paper to the influence of fatty acids on this transport system. The paper, however, describes the effects of fatty acids on other transport systems as well, such as Na^+-glucose, Na^+-amino acid and H^+-dipeptide cotransport systems.

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† Abbreviations: EGTA, ethylene glycol bis-(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; and Tris, Tris(hydroxymethyl)aminomethane.

MATERIALS AND METHODS

Materials. Free fatty acids, D-glucose, D-mannitol, Tris† base, MES, and HEPES were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. Carrier-free $^{22}NaCl$ (200 μCi/ml) and [U-^{14}C]-

glycylsarcosine (100 mCi/mmol) were from the Radiochemical Center, Amersham, England. D-[1-^{3}H-(N)]Glucose (15.5 Ci/mmol), L-[1-^{14}C]glucose (54.8 mCi/mmol) and L-[^{14}C-(U)]alanine (170 mCi/mmol) were purchased from New England Nuclear, Boston, MA, U.S.A. Dimethylamiloride was a gift from Edward J. Cragoe, Jr., Merck Sharp & Dohme Research Laboratory, West Point, PA, U.S.A. All other chemicals used were of analytical grade.

Preparation of brush-border membrane vesicles. Rabbit small intestine brush-border membrane vesicles were prepared by Mg^{2+} precipitation in the presence of EGTA [24]. After the rabbits were killed by a lethal dose of Nembutal, the entire small intestine was removed and rinsed with ice-cold 0.9% saline. The intestine was cut open longitudinally. The mucosa was scraped off and homogenized in 20 vol. (v/w) of ice-cold 150 mM mannitol, 2.5 mM EDTA, 6 mM Tris/NaOH buffer, pH 7.5, for 90 sec using a Waring blender. To this homogenate, 1 M $MgCl_2$ was added to a final concentration of 10 mM, and the mixture was stirred for 1 min and allowed to stand for 15 min. It was then centrifuged at 3000 *g* for 10 min. The supernatant fraction was centrifuged at 42,000 *g* for 30 min. The pellet was suspended in preloading buffer, consisting of 300 mM mannitol, 30 mM MES, 9 mM Tris, and 10 mM potassium gluconate, pH 5.5. This suspension was again centrifuged at 42,000 *g* for 30 min, and the pellet was resuspended in the same buffer using a syringe with a 25-gauge needle. In the final brush-border membrane preparation, protein concentration was determined, adjusted to 10–12 mg/ml, and used for Na^+ uptake measurement. The purity of brush-border membranes was assessed by assaying the marker enzyme alkaline phosphatase [14, 15]. The alkaline phosphatase activity was enriched 13- to 15-fold in the brush-border membranes compared to the homogenate.

For D-glucose, L-glucose and L-alanine transport studies, brush-border membrane vesicles were preloaded with a buffer containing 15 mM KCl, 270 mM mannitol, 10 mM HEPES, and 16 mM Tris, pH 7.5. Glycylsarcosine uptake was carried out in membrane vesicles that were preloaded with a buffer containing 100 mM K_2SO_4, 50 mM HEPES, 75 mM Tris, pH 8.4.

Transport studies. The uptake measurements were carried out by a rapid filtration technique [21] using Millipore filters (pore size, 0.65 μm). The specific conditions for each experiment are given in the respective figure legends. In general, uptake was initiated by addition of 40 μl of the membrane suspension containing 0.4 mg of membrane protein to 160 μl of uptake buffer. The mixture was shaken gently at 25° in a gyratory water bath shaker. At the end of the incubation period, uptake was stopped by adding 3 ml of ice-cold stop buffer and then it was filtered rapidly through a Millipore filter. The filter was washed three times with 5 ml of the stop buffer and then transferred to a counting vial. The radioactivity associated with the filter was measured by liquid scintillation spectrometry. Different stop buffers were used based on the uptake measurements. The stop buffers used were: 18 mM HEPES, 12 mM Tris, 163 mM KCl, pH 7.5, for Na^+ uptake; 11 mM HEPES, 10 mM Tris, 153 mM NaCl, pH 7.5, for D-glucose, L-glucose and L-alanine uptake; and 210 mM KCl, 2 mM HEPES, 2 mM Tris, pH 7.5, for glycylsarcosine uptake.

Fatty acid inhibition studies were carried out by adding fatty acids to the brush-border membrane as solutions of 100 times the final concentration in 90% ethanol [4, 25, 26]. Control experiments were done by adding equal concentrations of ethanol alone to the brush-border membranes. Brush-border membrane vesicles were preincubated with fatty acids at desired concentrations for 15–20 min, and then the vesicles were used for uptake measurements.

Uptake measurements were routinely done in duplicate or triplicate, and the variation between replicate values was always less than ±10% of the mean value.

RESULTS

Effect of fatty acids on proton gradient-coupled Na^+ uptake. Uptake of Na^+ (0.5 mM) was measured in intestinal brush-border membrane vesicles in the presence of an outward-directed proton gradient ($[pH]_i = 5.5$; $[pH]_o = 7.2$). Figure 1 shows that the time course of Na^+ uptake exhibited the overshoot phenomenon, indicating transient accumulation of Na^+ inside the vesicles against a concentration gradient. Figure 1 also shows that the H^+ gradient-dependent Na^+ uptake was inhibited drastically by oleic acid (0.5 mM). However, the equilibrium uptake

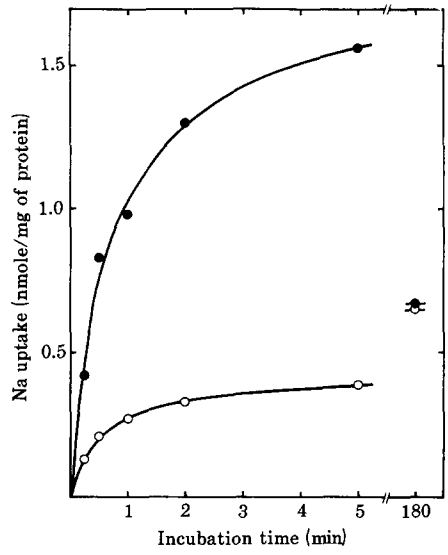

Fig. 1. Effect of oleic acid on intestinal Na^+-H^+ exchanger. Brush-border membrane vesicles were suspended in 300 mM mannitol, 30 mM MES, 9 mM Tris and 10 mM potassium gluconate, pH 5.5. Uptake was initiated by mixing 40 μl of membrane suspension (0.4 mg protein) with 160 μl of uptake buffer (300 mM mannitol, 18 mM HEPES, 18 mM Tris, 10 mM potassium gluconate, pH 7.5, containing 0.5 mM NaCl and traces of labeled Na^+). Under these conditions, the intravesicular pH was 5.5 and the extravesicular pH was 7.2. Treatment of membrane vesicles with oleic acid is described in Materials and Methods. Key: (●) control; and (○) in the presence of 0.5 mM oleic acid.

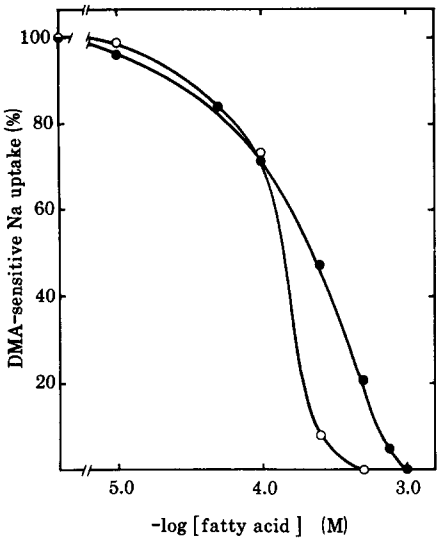

Fig. 2. Concentration-dependent inhibition of Na^+-H^+ exchanger by oleic acid and ricinoleic acid. Uptake of Na^+ (0.5 mM) was measured in brush-border membrane vesicles at 22° for 45 sec in the presence of outward-directed proton gradient. Concentrations of oleic and ricinoleic acids were varied from 0.01 to 1.0 mM. The uptake values represent only dimethylamiloride-sensitive Na^+ uptake. Key: (●) oleic acid; and (○) ricinoleic acid.

remained the same in the presence and absence of oleic acid, showing that oleic acid at a concentration of 0.5 mM did not alter the intravesicular volume.

Amiloride, a potassium-sparing diuretic, has been widely used to inhibit Na^+-H^+ exchanger activity. However, the sensitivity of the exchanger to amiloride inhibition varies from tissue to tissue. The ileal Na^+-H^+ exchanger is less sensitive to inhibition by amiloride than the renal Na^+-H^+ exchanger, and amiloride concentrations as high as 10 mM are needed to cause 85% inhibition of Na^+ uptake in ileal brush-border membrane vesicles [15]. In recent years, many derivatives of amiloride have been shown to be more effective inhibitors of the exchanger. In this study, we used dimethylamiloride (DMA) to inhibit the intestinal Na^+-H^+ exchanger. DMA at a concentration of 2 μM produced 50% inhibition of Na^+ uptake, whereas more than 95% inhibition was observed at 2 mM (data not shown). The uptake of Na^+ that occurs specifically via the Na^+-H^+ exchanger in intestinal brush-border membrane vesicles was therefore determined by subtracting Na^+ uptake measured in the presence of 2.5 mM DMA from the total uptake measured in the absence of the inhibitor.

Figure 2 illustrates the concentration-dependent inhibition of DMA-sensitive Na^+ uptake by oleic acid and ricinoleic acid. Ricinoleic acid, the active principle of castor oil, was found to be more inhibitory than oleic acid. Ricinoleic acid caused 50% inhibition at a 150 μM concentration, whereas oleic acid produced a 50% inhibition at 225 μM.

It was not clear from the above experiments whether the inhibition of the Na^+-H^+ exchanger was due to a direct interaction of the fatty acids with the exchanger protein or if it was an indirect effect owing to enhanced collapse of the H^+ gradient, the driving force for the exchanger system. We therefore compared the effects of oleic acid on the DMA-sensitive Na^+ uptake in the absence of a H^+ gradient ($[pH]_i = [pH]_o = 7.5$) and in the presence of a H^+ gradient ($[pH]_i = 5.5$; $[pH]_o = 7.2$). The initial rate of Na^+ uptake in the absence of a H^+ gradient was 2- to 3-fold less than in the presence of a H^+ gradient. Nonetheless, treatment of the membrane vesicles with oleic acid inhibited the Na^+ uptake to a similar extent in both the presence and absence of a H^+ gradient (Fig. 3). These results indicate that the inhibition of the Na^+-H^+ exchanger by oleic acid was not due to enhanced collapse of the H^+ gradient, but rather to a direct interaction of the fatty acid with the exchanger protein.

To determine the nature of inhibition, the effects of oleic acid on the kinetic parameters of the Na^+-H^+ exchanger were investigated. Proton gradient-stimulated Na^+ uptake was measured at various concentrations of Na^+ (1, 2.5, 5, 10 and 15 mM) in the absence and presence of 0.2 mM oleic acid. Incubation for 45 sec was used for the measurement of the initial rates of Na^+ uptake, because Na^+ uptake was found to be linear at least up to 45 sec (Fig. 1). The results are given in Fig. 4 as Eadie–Hofstee plots (v/s vs v). Oleic acid (0.15 mM) did not alter the apparent affinity of the exchanger for Na^+ (affinity constant, 6.5 mM), whereas the maximal velocity was reduced markedly (10.8 nmol/mg protein/45 sec vs 8.0 nmol/g protein/45 sec). These data suggest that the inhibition caused by oleic acid is non-competitive in nature with respect to Na^+.

The reversibility of oleic acid inhibition of the Na^+-H^+ exchanger was then studied. The brush-border membrane vesicles were preincubated with different concentrations of oleic acid and diluted 10-

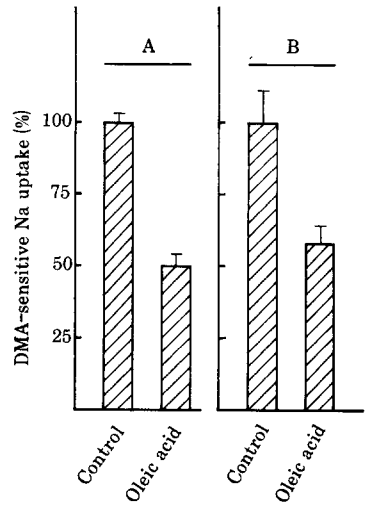

Fig. 3. Effects of oleic acid on the Na^+-H^+ exchanger in the presence and absence of a H^+ gradient. DMA-sensitive Na^+ uptake was measured with 45-sec incubations in control and oleic acid-treated membrane vesicles in the presence ($[pH]_o = 7.2$; $[pH]_i = 5.5$) (A) and absence ($[pH]_o = [pH]_i = 7.5$) (B) of a H^+ gradient. The concentration of oleic acid was 225 μM. The data are given as the mean ± SD for six determinations.

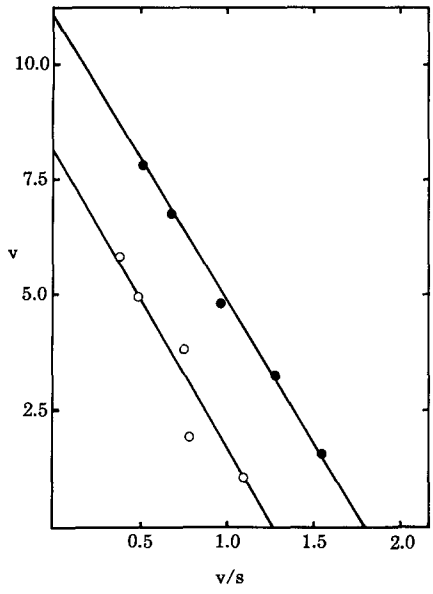

Fig. 4. Kinetics of oleic acid inhibition. Proton gradient-dependent Na^+ uptake was measured in brush-border membrane vesicles for 45 sec at various concentrations of Na^+ (1, 2.5, 5, 10, 15 mM) in the absence and in the presence of 0.15 mM oleic acid. The results are given as Eadie–Hofstee plots (v/s vs v). DMA-sensitive Na^+ uptake was used to construct the plots. Key: v, nmol of Na^+ uptake/mg protein/45 sec; s, Na^+ concentration (mM); (●) control; and (○) oleic acid.

fold in the presence of absence of oleic acid with uptake buffer, and Na^+ uptake was measured as described in Table 1. When the oleic acid concentration was 0.1 mM during preincubation as well as during the uptake, Na^+ uptake was inhibited by 25% compared to control. The inhibition increased to 92% when the concentration of oleic acid was 1.0 mM during preincubation and uptake. However, when the vesicles preincubated with 1.0 mM oleic acid were used to measure Na^+ uptake with the concentration of the oleic acid diluted to 0.1 mM during the uptake experiment, Na^+ uptake was inhibited by only 45%. If the inhibition by oleic acid had been irreversible, Na^+ uptake should have been inhibited to the same extent (i.e. 92%) in membrane vesicles that have been pretreated with 1 mM oleic acid, irrespective of the oleic acid concentration during uptake measurement. These results indicate that the inhibition of the intestinal Na^+-H^+ exchanger by oleic acid is partially reversible.

The influence of various long chain fatty acids on intestinal brush-border membrane Na^+-H^+ exchanger was studied, and it was found that the exchanger was inhibited 65–95% in the presence of 0.4 mM unsaturated fatty acids (Table 2). Of the saturated fatty acids tested, lauric acid (50%) and myristic acid (20%) showed considerable inhibition. Ricinoleic acid, a hydroxy unsaturated fatty acid, was found to be a more potent inhibitor of the Na^+-H^+ exchanger than the other fatty acids.

Effects of oleic acid on other transport systems. In addition to the Na^+-H^+ exchanger, we also tested the influence of oleic acid on other carrier-mediated as well as diffusional processes in brush-border membrane vesicles. For carrier-mediated systems, we chose to study Na^+-dependent transport of D-glucose and L-alanine and H^+-dependent transport of glycylsarcosine. Changes in diffusional properties of the membrane were studied by investigating the effect of oleic acid on the transport of L-glucose. All three carrier-mediated transport systems were markedly inhibited by oleic acid (Figs. 5 and 6). Interestingly, transport of L-glucose into these vesicles was not affected by oleic acid (Fig. 7).

To understand the mechanism of the inhibition of Na^+ gradient-driven transport systems by fatty acids, we investigated the effect of oleic acid on the Na^+-dependent uptake of L-alanine in the presence ($[Na^+]_o = 120$ mM; $[Na^+]_i = 0$ mM) and in the absence ($[Na^+]_o = [Na^+]_i = 120$ mM) of a Na^+ gradient. The data are given in Fig. 8. In the presence of a Na^+ gradient, oleic acid markedly inhibited L-alanine uptake. But, when measured in the absence of a Na^+ gradient, L-alanine uptake was not affected significantly by oleic acid. However, carrier-mediated L-alanine uptake did occur under these conditions because unlabeled L-alanine competed with labeled

Table 1. Reversibility of the inhibition of the intestinal Na^+-H^+ exchanger by oleic acid

Oleic acid concentration (mM)		Na^+ uptake	
During preincubation	During uptake	(nmol/mg protein/45 sec)	%
0	0	1.64 ± 0.10	100
0.1	0.1	1.23 ± 0.03	75
1.0	1.0	0.13 ± 0.05	8
1.0	0.1	0.90 ± 0.04	55

Membrane vesicles preloaded with pH 5.5 buffer (300 mM mannitol, 30 mM MES, 9 mM Tris, 10 mM potassium gluconate) were used. Forty microliters of membrane vesicle (0.4 mg protein) suspension was preincubated for 15 min at 22° (1) 10 μl of preloading buffer, (2) 10 μl of 0.5 mM oleic acid in preloading buffer, or (3) 10 μl of 5 mM oleic acid in preloading buffer. After the preincubation, 45-sec Na^+ uptake (0.5 mM) was measured by incubating these vesicles with either 450 μl of the uptake buffer or 450 μl of the uptake buffer containing 0.1 mM or 1.0 mM oleic acid. The composition of uptake buffer was: HEPES, 18 mM; Tris, 18 mM; potassium gluconate, 10 mM; mannitol, 300 mM; pH 7.5. The results are given as the mean ± SD for three determinations.

Table 2. Inhibition of intestinal brush-border membrane Na^+-H^+ exchanger by fatty acids

Fatty acids	Chain length	Na^+-uptake (nmol/mg protein/45 sec)	% Activity
None	—	1.25 ± 0.10	100
Octanoic	8:0	1.20 ± 0.03	96
Lauric	12:0	0.63 ± 0.05	50
Myristic	14:0	1.00 ± 0.04	80
Palmitic	16:0	1.15 ± 0.05	92
Stearic	18:0	1.20 ± 0.03	96
Oleic	18:1	0.44 ± 0.10	35
Linoleic	18:2	0.19 ± 0.03	15
Linolenic	18:3	0.13 ± 0.02	10
Ricinoleic	18:1—OH	0.06 ± 0.01	5

Different fatty acids at a concentration of 0.4 mM were treated with brush-border membrane vesicles, and Na^+ uptake was subsequently measured in these vesicles with 45-sec incubations. The experimental procedure for uptake measurements is given in the legend to Fig. 1. The results are given as the mean ± SD for three determinations.

L-alanine for the uptake process. Similar results were obtained when the Na^+-dependent uptake of D-glucose was studied (data not shown). These data suggest that the inhibitory effects of oleic acid on the Na^+ gradient-driven transport of D-glucose and L-alanine were due to enhanced collapse of the Na^+ gradient rather than a direct interaction with the carrier proteins.

The effect of oleic acid (0.01 to 1.0 mM) on brush-border membrane hydrolytic enzymes such as sucrase-isomaltase, maltase, trehalase, γ-glutamyl transferase and alakaline phosphatase was studied. Oleic acid did not inhibit any of these enzyme activities (data not shown).

DISCUSSION

The data presented in this paper demonstrate that treatment of rabbit intestinal brush-border membranes with fatty acids significantly affects, either directly or indirectly, the function of various transport systems in these membranes. The transport systems investigated here were D-glucose-Na^+ cotransport, L-alanine-Na^+ cotransport, dipeptide-H^+ cotransport and Na^+-H^+ exchanger. The Na^+-dependent transport of D-glucose and of L-alanine was inhibited by fatty acids only when the transport was assayed in the presence of an inward-directed Na^+-gradient. Fatty acids failed to show any inhibitory effect on these transport systems when there was no Na^+ gradient ($[Na^+]_i = [Na^+]_o$). Thus, the

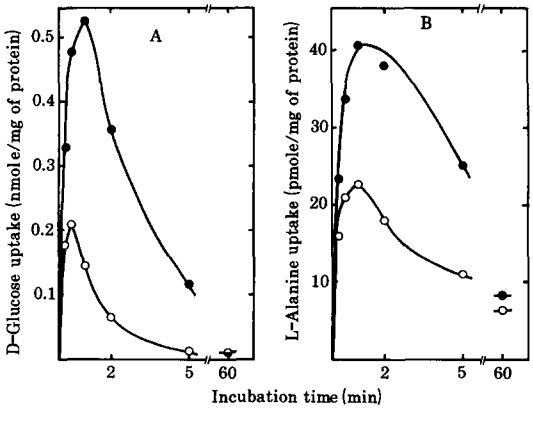

Fig. 5. Effect of oleic acid on Na^+-dependent transport systems. Brush-border membrane vesicles were preloaded with 15 mM KCl, 270 mM mannitol, 10 mM HEPES and 16 mM Tris buffer, pH 7.5. Uptake of 10 μM [^{3}H]D-glucose or 10 μM [^{14}C]L-alanine was measured in the presence of an inward-directed Na^+-gradient ($[Na^+]_o$ = 120 mM; $[Na^+]_i$ = 0). (A) Time course of D-glucose uptake. (B) Time course of L-alanine uptake. Key: (●) control; and (○) in the presence of 0.5 mM oleic acid.

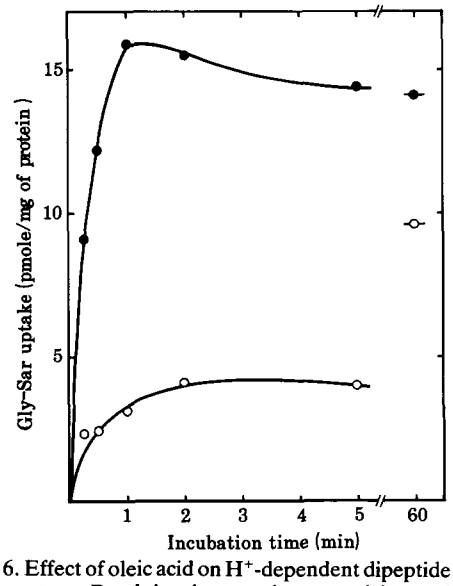

Fig. 6. Effect of oleic acid on H^+-dependent dipeptide transport system. Brush-border membrane vesicles preloaded with 100 mM K_2SO_4, 50 mM HEPES, and 75 mM Tris, pH 8.4, were used. Uptake of 20 μM [1-^{14}C]glycylsarcosine was measured after addition of 40 μl of membrane suspension (0.4 mg protein) to 160 μl of uptake buffer (50 mM MES, 50 mM HEPES, 25 mM Tris and 300 mM mannitol, pH 5.6). At the initiation of uptake, the intravesicular pH was 8.4 and the extravesicular pH was 6.7. Key: (●) control; and (○) in the presence of 0.5 mM oleic acid.

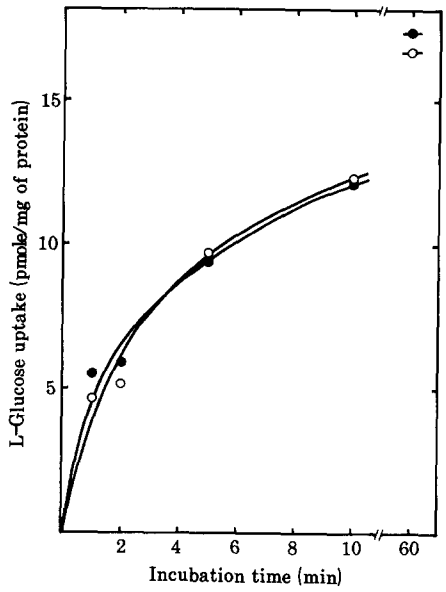

Fig. 7. Effect of oleic acid on non-carrier-mediated L-glucose uptake. The concentration of [1-^{14}C]L-glucose was 20 μM. The conditions for uptake measurements were similar to D-glucose uptake described in the legend for Fig. 5. Key: (●) control; and (○) in the presence of 0.5 mM oleic acid.

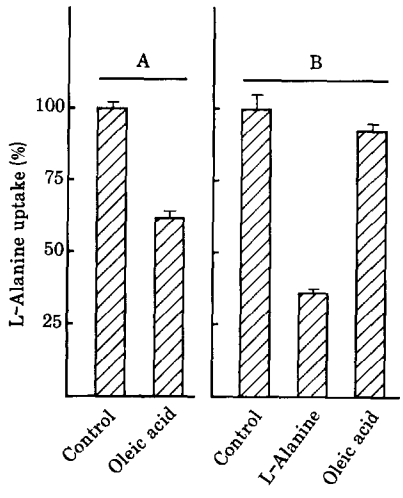

Fig. 8. Effect of oleic acid on L-alanine uptake in the presence and absence of a Na^+ gradient. Uptake of radiolabeled L-alanine (10 μM) was measured with 30-sec incubations in control and oleic acid-treated membrane vesicles in the presence ($[Na^+]_o = 120$ mM, $[Na^+]_i = 0$) (A) and absence ($[Na^+]_o = [Na^+]_i = 120$ mM) (B) of a Na^+ gradient. The concentrations of oleic acid and unlabeled L-alanine were 0.5 and 5 mM respectively. In the presence of a Na^+ gradient (A), 100% uptake corresponded to 33.7 ± 0.9 pmol/mg protein/30 sec. In the absence of a Na^+ gradient (B), 100% uptake corresponded to 12.2 ± 0.6 pmol/mg protein/30 sec. The data are given as the mean ± SD for six determinations.

inhibition of the D-glucose-Na^+ cotransport or L-alanine-Na^+ cotransport by fatty acids in isolated brush-border membrane vesicles appears to be due to the enhanced collapse of the Na^+ gradient rather than a direct effect on the transport systems. On the contrary, the activity of Na^+-H^+ exchanger was inhibited by fatty acids in both the presence and absence of a H^+ gradient, suggesting that the inhibition was due to a direct effect on the transport system. Since the Na^+-H^+ exchanger as well as the dipeptide transport system are driven by a H^+ gradient (though the directions of the gradient are different) in intestinal brush-border membrane vesicles and since there was no indication of fatty acid-induced collapse of the H^+ gradient, we conclude that the treatment of the brush-border membranes with fatty acids directly interferes with the function of these transport systems.

Additional experiments on the fatty acid-induced inhibition of the intestinal Na^+-H^+ exchanger revealed that unsaturated fatty acids were more inhibitory than saturated fatty acids. The greater inhibition by unsaturated fatty acids as compared to saturated ones shows that the presence of a more fluid acyl chain is essential for interaction with the membrane. It has been shown previously that unsaturated fatty acids are more potent than saturated fatty acids in inhibiting (Na^+-K^+)ATPase [4] and several other membrane-bound enzymes [25, 26]. However, it is possible that the lack of effect of the saturated long chain fatty acids (C-16 and C-18) may be related to their relative insolubility in the aqueous medium compared to the unsaturated fatty acids.

The mechanism by which the inhibitory fatty acids exert their effects on the Na^+-H^+ exchanger is not clear. Kinetic analyses with oleic acid showed that the fatty acid-induced inhibition was non-competitive in nature. Moreover, the inhibition was only partially reversible. The equilibrium uptake of various solutes into the vesicles and the activities of many brush-border membrane hydrolases were not altered by the treatment of the membrane vesicles with fatty acids, strongly suggesting that the treatment, under the experimental conditions employed in the present study, did not affect the integrity of the membrane lipid bilayer, intravesicular volume, and the membrane surface properties. It is likely that the interaction of the brush-border membranes with oleic acid results in significant changes in the physical properties of the membranes which may interfere with the catalytic function of many, if not all, transport systems. It has been observed previously that the addition of oleic acid to the membranes induces a decrease in the Arrhenius plot break-point temperature and the activation energy of membrane-bound ATPases [26, 27]. Merrill *et al.* [28] have shown recently that incubation of rabbit intestinal brush-border membrane vesicles with oleic acid leads to rapid incorporation of the fatty acid into the membranes and thereby induces an increase in the fluidity of the brush-border membranes. It is therefore possible that the inhibitory fatty acids affect the physical state of the brush-border membranes and subsequently interfere with the function of certain transport proteins.

The present investigation demonstrates that many

fatty acids, particularly unsaturated fatty acids and hydroxy fatty acids, inhibit the Na^+-H^+ exchanger in rabbit intestinal brush-border membrane vesicles. Inhibition of the exchanger by oleic acid is noteworthy because this fatty acid has been shown to be a major constituent of fecal unsaturated fatty acids [4]. In addition, ricinoleic acid, a known laxative, is one of the most potent inhibitory fatty acids. In contrast to the present studies where the Na^+-H^+ exchanger is driven by an experimentally imposed H^+ gradient, the Na^+-H^+ exchanger *in vivo* is driven by a Na^+ gradient. Even though the present studies have demonstrated that many unsaturated fatty acids directly interfere with the activity of the exchanger, they also increase the permeability of the brush-border membrane to Na^+. This increase in the Na^+ permeability is probably the mechanism of fatty acid-induced inhibition of glucose and amino acid transport, observed both *in vitro* as well as *in vivo* [7–10]. Such a collapse of the Na^+ gradient across the brush-border membrane *in vivo* caused by the unabsorbed fatty acids in the intestinal lumen would also result in the decreased activity of the Na^+-H^+ exchanger.

It is difficult at this time to assess the relevance of the *in vitro* demonstration of inhibition of the brush-border membrane Na^+-H^+ exchanger by free fatty acids to the *in vivo* observation that the presence of excess fatty acids in the intestinal lumen induces diarrhea. Nonetheless, some speculations can be made. Under physiological conditions, the brush-border membrane Na^+-H^+ exchanger and the basal-lateral membrane (Na^+–K^+)ATPase are responsible for the generation and maintenance of ion gradients across the brush-border membrane, and these ion gradients energize many active transport systems. Impairment of either the Na^+-H^+ exchanger or the (Na^+–K^+)ATPase or both would lead to decreased absorption of organic solutes and inorganic ions whose transport systems are dependent on a Na^+-gradient or a H^+-gradient. This would, in turn, induce osmotically-coupled water excretion in feces resulting in diarrhea. The present findings on the inhibition of the Na^+-H^+ exchanger by fatty acids together with a previous report [4] describing a similar phenomenon in the case of (Na^+–K^+)ATPase suggest that these inhibitory effects of unabsorbed fatty acids in the lumen of intestine could play a significant role in the pathogenesis of fatty acid-induced diarrhea.

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