Characteristics of Lysophosphatidylcholine in Its Inhibition of Taurine Uptake by Human Intestinal Caco-2 Cells

Koji ISHIZUKA, Yusei MIYAMOTO,* Hideo SATSU, Ryuichiro SATO, and Makoto SHIMIZU†

Department of Applied Biological Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan *Department of Integrated Life Sciences, The University of Tokyo, Kashiwa-shi, Chiba 277-8562, Japan

Received August 30, 2001; Accepted December 9, 2001

The characteristics of lysophosphatidylcholine (LPC) in its inhibition of the taurine uptake by human intestinal Caco-2 cells were investigated. By treating the cells with 200 μM of LPC, the taurine uptake was rapidly decreased by approximately 60%. This decrease was accompanied by an increase in the K_m value for the uptake. A rapid uptake of LPC itself by the cells was also observed. The inhibitory activity of LPC was specific to the uptake of taurine and certain amino acids, while the uptake of glucose, glutamic acid and peptide (glycylglutamine) was not affected by LPC. The activity was dependent on the structure of a polar head and the bound fatty acid. The phosphorylcholine residue was likely to have played an important role, and surface active LPC with fatty acids of C14 or longer was highly inhibitory. These results suggest that the interaction of LPC with the taurine transporter in the intestinal cell membrane was the cause of the reduced taurine uptake.

Key words: taurine; Caco-2; small intestine; transporter; lysophosphatidylcholine

Taurine is known as an important nutrient that is required for a number of biological processes, including antioxidation, anti-inflammation, detoxification and osmoregulation.1−3 Although human beings possess the biosynthetic capability for taurine, the biosynthetic activity is not sufficiently high when compared with that of other animal species.4) The uptake of taurine from dietary sources is therefore important to maintain the taurine level in cells and tissues, particularly for infants and patients with inflammatory diseases. The intestinal taurine transporter (TAUT) is thought to be responsible for the uptake of dietary taurine.5) TAUT is a membrane protein with 12 transmembrane domains, and is expressed in almost all tissues. We have characterized intestinal TAUT expressed in the human intestinal Caco-2 cell, and found that TAUT was adaptively regulated by various external factors; e.g., osmotic stress6,7) and the taurine concentration surrounding the cells.6,8) These two factors affected the expression of TAUT at least at the transcriptional level. In addition to the regulation being dependent on the intracellular signaling mechanism, regulation of the transporter activity via the direct effect of extrinsic factors on the transporter molecules could also occur.

Small intestinal epithelial cells express many nutrient transporters such as those for glucose (SGLT1 and GLUTs),9) amino acid10) and peptide (PepT1).11) Since these transporters expressed on the apical side of the epithelial cell layers are always exposed to various food substances in the intestinal tract, their functions might be affected by food components. We have recently found that the activity of SGLT1, the major glucose transporter in the small intestine, was inhibited by such food substances as tea polyphenols,12) the inhibition by tea polyphenols being performed in a competitive manner. This suggests that some food substances could play a role as regulatory factors of intestinal transporters. Those substances may interact with the intestinal transporters or with transporter-associated molecules, thus affecting the intestinal absorption of nutrients.

To examine the effects of food substances on intestinal TAUT, we have reported a search in the previous paper for food factors that could enhance or suppress the TAUT activity.13) A factor that inhibited taurine uptake by Caco-2 cells was detected in an alcohol extract of black sesame seeds, and the active substance was identified as lysophosphatidylcholine (LPC) with linoleic acid. The inhibition of taurine uptake by LPC was dose-dependent, the IC_50 value of the effect being about 200 μM.13) The inhibition by LPC took place rapidly, maximum inhibition being observed after incubating the cells with LPC for only 5 min. Studies on the characteristics and mechanism for this inhibitory effect are reported in this present paper.

Materials and Methods

Materials. The Caco-2 cell line was obtained from...
the American Type Culture Collection (Rockville, MD, U.S.A.). Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Nissui Pharmaceuticals (Tokyo, Japan). L-glutamine and penicillin-streptomycin (10,000 units/ml and 10 mg/ml in 0.9% sodium chloride, respectively) were purchased from Gibco (Gaithersburg, MD, U.S.A.). Fetal bovine serum (FBS) was purchased from Iwaki Glass (Chiba, Japan), and non-essential amino acids (NEAA) were purchased from Cosmobi (Tokyo, Japan). A type-1 collagen solution was obtained from Nitta Gelatin (Osaka, Japan), and a Hank’s balanced salt solution (HBSS) was from Life Technologies (Grand Island, NY, U.S.A.). [1,2-3H]-taurine (specific radioactivity, 19 Ci/mmol), 1-[4,5-3H]-leucine (specific radioactivity, 142 or 167 Ci/mmol), 1-[4,5-3H]-lysine (specific radioactivity, 46 Ci/mmol), 4-[3H]-glutamic acid (specific radioactivity, 46 Ci/mmol) and 1-[6-3H]-glucose (specific radioactivity, 33 Ci/mmol) were purchased from Amersham (Little Chalfont, England), and [3H]-glycyl-glutamine (specific radioactivity, 4 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA, U.S.A.). Phosphatidylcholines (PCs; C18:2, C18:1 and C18:0), lysophosphatidylcholines (LPCs; C18:1, C18:0, C14:0, C12:0 and C10:0), lysophosphatidyleserine (LPS; C18:0) and soybean lysophosphatidylinositol (LPI) were obtained from Sigma (St. Louis, MO, U.S.A.). LPC (C18:2), l-lysophosphatidic acid (LPA; C18:1) and linolenic acid were purchased from Funakoshi (Tokyo, Japan), and hens’ egg lysophosphatidylethanolamine (LPE) was purchased from ICN Biomedicals (Costa Mesa, CA, U.S.A.). Phosphorycholine was from Tokyo-Kasei (Tokyo, Japan). Sugar esters (succrose monostearate; S370, S770 and S1170), monoglycer estearate (Excel T95) and pentaglycer estearate (PGMS) were from Etsuki Glico Co. (Osaka, Japan), the HLB values for S370, S770, S1170, Excel T95 and PGMS being 3, 7, 11, 3 and 13, respectively. All other chemicals were used as of reagent grade.

Cell culture. Caco-2 cells were cultured in 78.5-cm² plastic dishes with a culture medium consisting of DMEM, 10% FBS, 1% NEAA, 2% glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin and an appropriate amount of sodium bicarbonate. The cells were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air, the culture medium being renewed on alternate days. After they had reached confluence, the cells were passaged at a split ratio of 4 to 8 by trypsinizing with 0.1% trypsin and 0.02% EDTA in phosphate-buffered saline (PBS). All the cells used in this study were between passages 40 and 85. The uptake experiment was performed by using Caco-2 cells that had been cultured at a density of 1.4 × 10⁵ cells/well in 24-well plates precoated with collagen. The cell monolayers for the uptake experiments were used after 14 days of culture.

Uptake experiments. Uptake experiments on the radio isotope-labeled nutrients were performed according to the method of Satsu et al. (25). Caco-2 cell monolayers were washed twice with 700 µl of HBSS containing 4 mM sodium bicarbonate and 10 mM HEPES, the pH value being adjusted to 7.4 with KOH. For the uptake experiment on dipeptide (Gly-Gln), HBSS of pH 6.0 was used throughout the experiment because the peptide transporter is proton-dependent. The cells were then incubated with 300 µl of HBSS with or without an assay sample at 37°C for 30 min. The sample solution was then removed, and each well was washed with 700 µl of HBSS before the cells were incubated with each of the tritium-labeled nutrients in 300 µl of HBSS at 37°C for 10 min. At the end of the incubation period, the buffer was removed, and each monolayer was carefully washed three times with 700 µl of ice-cold PBS containing 0.05% sodium azide for 5 min. To each well was then added 250 µl of 0.1% Triton X-100, and the cell lysate was taken into 3 ml of a scintillation cocktail. The tritium content of each monolayer was finally determined with an LSC 5100 liquid scintillation analyzer (Aloka, Tokyo, Japan).

Kinetic analysis of the taurine uptake. The taurine uptake was measured over a concentration range of 0.5-25 µM. Lineweaver-Burk reciprocal plots were constructed to calculate the Vₘₐₓ and Kₘ values for taurine transport in the Caco-2 cells.

Measurement of the surface tension. The surface tension, expressed in dyne/cm, was measured with a Wilhelmy plate-type surface tensiometer (model CBVP-A3, Kyowa Kagaku, Tokyo, Japan) by using a platinum plate. Each sample solution was adjusted to pH 7, and the surface tension was measured at room temperature.

Statistical analysis. Each result is expressed as the mean ± SE. Differences between the experimental and control data were assessed by Student’s t test.

Results

Characteristics of the taurine uptake inhibition by LPC

In the present experiments, 200 µM of LPC was used, unless otherwise stated, because the IC₅₀ value for TAUT inhibition by LPC was about 200 µM. To reveal the mechanism for the inhibition of taurine uptake by LPC, the effect of LPC on the kinetics of the taurine uptake by Caco-2 cells was analyzed. The LPC treatment increased the Kₘ value from 5.6 µM
(control cells) to 17.0 \(\mu M\) (LPC-treated cells), suggesting that the affinity of the taurine transporter in the Caco-2 cells was reduced by LPC. The inhibitory effect of LPC lasted for more than 60 min, even after the treated Caco-2 cells had been washed to remove free LPC (data not shown). This suggests that LPC tightly bound to or was incorporated into the cells, thereby inhibiting the taurine uptake. The content of LPC incorporated into the cells increased during incubation with LPC, indicating that LPC was taken into the cells in a time-dependent manner (Fig. 1). A rapid uptake of LPC was apparent during the initial 60 sec, suggesting that part of LPC in the aqueous phase could be quickly adsorbed to the surface of the cells. Good correlation between this LPC uptake and the ability of LPC to inhibit TAUT was observed.

The effects of LPC on the uptake of other amino acids, peptides and glucose by Caco-2 cells were also examined. Although the uptake of leucine and lysine was significantly inhibited by LPC, the taurine uptake was most markedly inhibited (Fig. 2). On the other hand, the uptake of glutamic acid, glycylglutamine and glucose was not significantly affected by LPC, indicating that the effect of LPC was specific to certain types of transporter.

**Analysis of the structural factors of LPC responsible for the taurine uptake inhibition**

LPC has one fatty acid chain and one charged group, phosphoryl-choline, in its molecule. To reveal the molecular structure of LPC that was essential for the inhibition of taurine uptake, we evaluated the effect of other lysophospholipids with different fatty acid chains or charged groups. As shown in Fig. 3, not only LPC (with linoleic fatty acids; C18:2) but also LPC with fatty acid chains larger than C14 showed the inhibitory effect. LPC with a fatty acid chain shorter than C12 did not show any inhibitory activity. The activity of LPC with saturated fatty acid (C18:0) was similar to that with unsaturated fatty acid (C18:2). On the other hand, lysophospholipids with other charged groups were observed to be inactive (Fig. 4). Phosphatidylcholine (C18:0, C18:1 and C18:2), phosphorylcholine and linoleic acid were also not inhibitory, although the treatment by phosphorylcholine resulted in slightly lower taurine uptake. These results indicate that a relatively long fatty acid chain and the phosphorylcholine residue as a charged group were necessary for the strong inhibitory activity of lysophospholipids.

**Correlation between the inhibition of taurine uptake and the surface activity of LPC**

In the present experiment, we observed that an aqueous solution/dispersion of a taurine-uptake-inhibiting lysophospholipid was likely to have good foaming properties. We therefore measured the surface tension of lysophospholipid solutions to exa-

![Fig. 1. Time-dependent Uptake of LPC by Caco-2 Cell Monolayers.](image)

Caco-2 cell monolayers were incubated with \(^{14}\)C-labelled LPC at a concentration of 200 \(\mu M\). At each indicated time, the cell monolayers were washed by HBSS, and the radioactivity incorporated into the cell monolayers was measured. Each value is the mean \(\pm S.E.\) (\(n = 5\)). The effect of the incubation time with LPC on the taurine uptake is also shown.

![Fig. 2. Effect of LPC on the Uptake of Nutrients by Caco-2 Cell Monolayers.](image)

Cells were precultured with 200 \(\mu M\) of LPC for 30 min, and the uptake of nutrients was then measured. Each value is the mean \(\pm S.E.\) (\(n = 6\)). *\(P < 0.05\) vs control.

![Fig. 3. Effect of LPC on the Uptake of Taurine by Caco-2 Cell Monolayers.](image)

Cells were precultured with 200 \(\mu M\) of LPC for 30 min, and the uptake of taurine was then measured. Uptake experiments were performed as described in the Materials and Methods section. Each value is the mean \(\pm S.E.\) (\(n = 6-11\)). *\(P < 0.05\) vs control.
Taurine Uptake Inhibition by Lysophosphatidylcholine

Fig. 4. Effect of LPCs and Their Related Compounds on the Uptake of Taurine by Caco-2 Cell Monolayers.
Cells were preincubated with 200 μM of LPC for 30 min, and the uptake of taurine was then measured. Each value is the mean ± S.E. (n = 8–11). *P<0.05 vs control.

Fig. 5. Correlation between the Taurine Uptake-modulatory Activity and the Surface Activity of LPCs and Their Related Compounds.
The sample concentration used for the analyses was 200 μM. Samples: (1) LPC, C18:2, (2) LPC, C18:1, (3) LPC, C18:0, (4) LPC, C14:0, (5) LPC, C12:0, (6) LPC, C10:0, (7) LPA, (8) LPS, (9) LPE, (10) LPI, (11) phosphorylcholine, (12) PC, 18:2, (13) PC, 18:1, (14) PC, 18:0, (15) HBSS.

mine the correlation between their surface activity and the taurine uptake inhibitory activity. As shown in Fig. 5, a positive correlation (r = 0.82) between these two properties was observed. This suggests that being surface active is at least one of the factors required for the taurine uptake inhibitory activity of lysophospholipids.

To reveal whether high surface activity was a necessary and sufficient condition for the inhibitory substance, we examined the effects of Triton X-100 and taurocholic acid as surfactants. Triton X-100 is a compound frequently used in biochemical experiments as a solubilizer or as a surface-active agent.

The hydrophilic-lipophilic balance (HLB) value and critical micellar concentration (CMC) of Triton X-100 are 14 and 0.24 mM, respectively, which are similar to those of LPC (13 and 0.20 mM). Triton X-100 was observed to inhibit the taurine uptake by Caco-2 cells, the effect being as rapid as that of LPC. The inhibitory effect of Triton X-100 was dose-dependent, and the IC50 value was 80 μM, at which concentration the surface tension was approximately 40 dyne/cm, corresponding to the surface tension of an LPC solution at its IC50 value (200 μM). However, Triton X-100 inhibited not only the uptake of taurine but also that of other nutrients such as glucose and glycylglutamine (Fig. 6). The inhibitory mechanism by Triton X-100 is therefore thought to have been different from that by LPC. On the other hand, sodium taurocholate did not show any inhibitory effect on the taurine uptake by Caco-2 cells (Fig. 7),
although a taurocholate solution of 200 \( \mu M \) or higher gave a surface tension value of 40 dyne/cm or lower. Furthermore, we observed that there was no correlation between the taurine uptake inhibitory activity and the surface tension for such emulsifiers as sugar esters and monoglycerides (Fig. 8). These results suggest that the mechanism for the taurine uptake inhibition by LPC was not simply due to its surface-active properties. Surface-active substances do not necessarily have an inhibitory effect on taurine uptake, although being surface active is likely to be a necessary characteristic that enhances the inhibitory activity of LPC.

**Effect of phosphorylcholine on the taurine uptake by Caco-2 cells**

Phosphorylcholine, the charged group of LPC, has a structure similar to that of taurine in terms of being zwitterionic. Therefore, the phosphorylcholine residue in LPC could play a role in the inhibition of taurine uptake; for example, it could be recognized by the taurine transporter as a competitive substrate. To evaluate this hypothesis, the uptake of taurine by Caco-2 cells was examined in the presence and absence of phosphorylcholine. The taurine uptake by Caco-2 cells was significantly lower (75\% of the control value) in the presence of 200 \( \mu M \) phosphorylcholine, although this inhibition was not significant when phosphorylcholine had been washed away before the uptake experiment (Fig. 4).

**Discussion**

The inhibition of taurine uptake in human intestinal Caco-2 cells by LPC was observed in this study. Since LPC is a well-known surface-active substance and a high concentration of LPC has cell lytic activity,\(^{16}\) it was speculated that the cellular damage caused by LPC would be the primary cause of this inhibition. However, LPC did not show any cytotoxic effect toward the Caco-2 cells at a concentration of 200 \( \mu M \) and lower.\(^{13}\) Therefore, this inhibition was likely to have been due to a specific action of LPC on the Caco-2 cell transport functions. This specificity is supported by the fact that the inhibition was observed only for TAUT and for some of the amino acid transporters, the activity of the glucose and peptide transporters not being affected by the LPC treatment (Fig. 2).

It has been reported that LPC has a variety of biological functions such as activation of endothelial cells,\(^{17}\) stimulation of macrophages\(^{18}\) and modulation of T-cell functions.\(^{19}\) Regulation of the transporter functions by LPC has also been reported by some researchers. Durante et al.\(^{20}\) have observed that LPC regulated cationic amino acid transport in vascular smooth muscle cells. The impairment of arginine transport in aortic endothelial cells by LPC has been reported by Kikuta et al.\(^{16}\) Intestinal functions could also be affected by LPC; for example, Muir et al.\(^{21}\) have reported that cholesterol synthesis in intestinal Caco-2 cells was increased by LPC. However, most of these cell modulatory functions of LPC are likely to be due to the transcriptional regulation of certain genes or to modulation of the signal transduction pathways. The increase in cholesterol synthesis in Caco-2 cells has, for example, been reported to be due to the increased gene expression of HMG-CoA reductase.\(^{22}\) Nishi et al.\(^{19}\) have reported that LPC-induced activation of T lymphocytes in atherogenesis was accompanied by the up-regulation in gene expression of the heparin-binding epidermal growth factor (HB-EGF). At least several hours are therefore needed to stimulate or suppress cell functions by these types of regulation.

On the other hand, the inhibition of TAUT by LPC that was observed in the present study was a very rapid process, occurring in less than 1 min.\(^{13}\) This suggests that direct interaction between LPC and TAUT was responsible for this inhibition. As shown in Fig. 1, the uptake of LPC by Caco-2 cells occurred in a time-dependent manner. The very rapid uptake of LPC observed in the first minute suggests that LPC was quickly adsorbed to the surface of the Caco-2 cells. The surface-active property of LPC may have played a role in this rapid adsorption. A good correlation between the surface activity and TAUT-inhibitory activity of lysophospholipids (Fig. 5), as well as the similar time-dependent characteristics in the cellular uptake of LPC and taurine-uptake inhibition (Fig. 1) suggest the adsorption of LPC to the Caco-2 cell surface or its binding to the surface molecules to have been responsible for the TAUT inhibition by LPC. The possible mechanism
for this is that LPC is first adsorbed to the surface of Caco-2 cells with the alkyl chain being inserted into the membrane lipid bilayer. The polar phosphorylcholine moiety of LPC then comes into contact with the substrate-binding site of TAUT, whereby the affinity of TAUT for taurine is reduced. The reason why the uptake of such amino acids as leucine and lysine was reduced by the LPC treatment is not known. A detailed analysis of the conformation and stability of these amino acid transporters in the future will answer this question. It may however be possible that the LPC-induced membrane fluidity change affected the conformation of these transporters, resulting in their dysfunction as will be described later.

Phosphorylethanolamine is structurally more like taurine than phosphorylcholine (an amino group instead of a trimethylamino group). However, LPE was not inhibitory to the taurine uptake (Fig. 4). This may be explained by the low surface activity of LPE (Fig. 5). Incorporation into the cell membrane is therefore thought to be very important for lysophospholipids to express their TAUT inhibitory activity. Changes in the cell membrane fluidity by the incorporation of LPC22 may also affect the activity of TAUT. Fujii et al.23 have reported that LPC inhibited glucose transporters in erythrocytes by changing the cell membrane properties. Regulation of the K+ transporter in gastric membranes by LPC through specific interaction has also been reported.24

Since the effect of LPC was observed with a very short incubation time, the effect on transcription and translation may be ruled out. However, other possibilities such as LPC-induced phosphorylation of TAUT cannot be ruled out, because LPC is capable of modulating second messenger signaling pathways.25 We have examined the effect of various inhibitors on the taurine-uptake inhibitory activity of LPC. Treatment of Caco-2 cells with pertussis toxin (G-protein inhibitor; 50 ng/ml), PD98059 (MAP kinase inhibitor; 10 μM), Wortmannin (PI-3 kinase inhibitor; 10 nM) or Et-18-OCH3 (PI-PLC inhibitor; 15 μM) did not affect the LPC-induced inhibition of TAUT (unpublished results). Second messenger signal pathways are therefore not likely to be involved in this inhibition.

Lambert and Falktof26 have recently reported that LPC (25 μM) induced taurine efflux from HelA cells, together with a loss of adenosine nucleotides and the induction of Ca2+ influx. They proposed a general breakdown of the membrane permeability barrier of HelA cells. Such an increase in the taurine efflux, if any, could partly contribute to the apparent decrease in taurine uptake by the Caco-2 cells. However, the rapid inhibition process induced by LPC,23 the specific inhibition of taurine uptake (Fig. 2) and the significant inhibition by phosphorylcholine suggest that the apparently low value for the taurine uptake activity in LPC-treated Caco-2 cells was not simply due to the increased taurine efflux. A reduction of the taurine uptake by specific interaction between LPC and TAUT, rather than any increased efflux, is thought to have been the major reason for the reduced amount of intracellular [3H]-taurine.

LPC is known to be present in the seeds of beans and cereals, and also in egg yolk.27 These phosphatidylcholine (PC)-rich food materials could be suppliers of LPC, because LPC is easily produced from PC by hydrolysis with phospholipase A2 that is secreted into the intestinal tract. Lipid-rich diets induce the intestinal secretion of bile juice which also contains phospholipids. The present results suggest that lipid (phospholipid)-rich diets may interfere with the intestinal uptake of taurine.

References