RESEARCH ARTICLE

Lipopolysaccharide inhibits colonic biotin uptake via interference with membrane expression of its transporter: a role for a casein kinase 2-mediated pathway

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Lipopolysaccharide inhibits colonic biotin uptake via interference with membrane expression of its transporter; a role for a casein kinase 2-mediated pathway. Am J Physiol Cell Physiol 312: C376–C384, 2017. First published January 4, 2017; doi:10.1152/ajpcell.00300.2016.—Biotin (vitamin B7), an essential micronutrient for normal cellular functions, is obtained from both dietary sources as well as gut microbiota. Absorption of biotin in both the small and large intestine is via a carrier-mediated process that involves the sodium-dependent multivitamin transporter (SMVT). Although different physiological and molecular aspects of intestinal biotin uptake have been delineated, nothing is known about the effect of LPS on the process. We addressed this issue using in vitro (human colonic epithelial NCM460 cells) and in vivo (mice) models of LPS exposure. Treating NCM460 cells with LPS was found to lead to a significant inhibition in carrier-mediated biotin uptake. Similarly, administration of LPS to mice led to a significant inhibition in biotin uptake by native colonic tissue. Although no changes in total cellular SMVT protein and mRNA levels were observed, LPS caused a decrease in the fraction of SMVT expressed at the cell surface. A role for casein kinase 2 (CK2) (whose activity was also inhibited by LPS) in mediating the endotoxin effects on biotin uptake and on membrane expression of SMVT was suggested by findings that specific inhibitors of CK2, as well as mutating the putative CK2 phosphorylation site (Thr78Ala) in the SMVT protein, led to inhibition in biotin uptake and membrane expression of SMVT. This study shows for the first time that LPS inhibits colonic biotin uptake via decreasing membrane expression of its transporter and that these effects likely involve a CK2-mediated pathway.

biotin; LPS; transport colon

BIOTIN (vitamin B7) is an essential micronutrient for normal human health because of its involvement in a variety of critical metabolic reactions. The vitamin acts as a cofactor for five carboxylases that are critical for fatty acid, glucose, and amino acid metabolism (36, 38, 46). Important roles for biotin in the regulation of cellular oxidative stress (35), gene expression [in which expression of over 2,000 human genes appears to be affected by biotin status (44, 55, 56)], and in normal function of the immune system (29) have also been recognized. In reference to the latter, biotin was shown to be important for activity, generation, maturation, and/or responsiveness of immune cells (5, 7, 28, 42); also deficiency of the vitamin has been shown to lead to an increase in the levels of proinflammatory cytokines like tumor necrosis factor-α, interleukin-1β, and interferon-γ (18, 30, 31). A role for biotin in the maintenance of normal intestinal integrity and homeostasis (18, 45), in influencing the colonization/invasiveness of certain enteropathogenic bacteria (57), and in mediating the effect of probiotic bacteria on gut microbial community (54) has, in addition, been recently reported. It is not surprising, therefore, that deficiency/suboptimal levels of biotin lead to disturbances in the normal function of many systems and ultimately to clinical manifestations (38, 46, 54). Deficiency/suboptimal levels of biotin occur in a variety of conditions including inflammatory bowel disease (IBD) (1, 14), inborn errors in biotin metabolism, multiple carboxylase deficiency (12), and chronic alcoholism (10).

Humans and other mammals cannot synthesize biotin and thus must obtain the vitamin from exogenous sources via intestinal absorption. The intestine is exposed to two sources of biotin, a dietary source (absorbed in the small intestine) and a microbiota-generated source (absorbed in the large intestine) (41, 48, 49). Absorption of biotin in both the small and large intestine occurs via a carrier-mediated process that involves the sodium-dependent multivitamin transporter (SMVT) system encoded by the SLC5A6 gene. This carrier system is exclusively expressed at the apical membrane domain of polarized absorptive epithelial cells (52). Studies from our laboratory and others have delineated different cell and molecular aspects of the intestinal biotin uptake process, how the process is regulated at the transcriptional and posttranscriptional levels, and how specific external (environmental)/internal factors and conditions affect and interfere with the event (46–49). Nothing, however, is presently known about the effect of the bacterial LPS on intestinal biotin uptake process, so it was therefore examined in this study.

LPS is a powerful bacterial virulence factor in terms of proinflammatory properties and is a source of considerable clinical morbidity and mortality. This endotoxin is a major component of the outer membrane of Gram-negative bacteria that is released from bacterial cell walls by shedding or through bacterial lysis and acts as a potent activator of the inflammatory response in the gut (27). The concentration of LPS is highest in the gut lumen (especially colonic lumen because it harbors a large collection of bacteria) and increases markedly in patients with IBD and in those infected with enteric pathogens (e.g., Salmonella) (8, 22, 51). LPS generated in the gut lumen exerts its effects on the lining epithelia via interaction with Toll-like
receptor-4 (TLR-4) (3, 4, 22). Although LPS has been shown to affect intestinal transport of a variety of substrates (2, 15, 37), its effect on the intestinal uptake of biotin has not been investigated. We addressed this issue in the present investigation using the human colonic epithelial NCM460 cells and mice as models. Our results showed that in vitro and in vivo exposure to LPS leads to a decrease in biotin uptake by colonic epithelial cells with no change in level of expression of total cellular SMVT protein or its mRNA. Rather the effect appears to be due to a decrease in membrane expression of the SMVT protein, likely mediated via a role for casein kinase 2 (CK2).

**Materials and Methods**

**Materials**

[^3]H-Biotin (specific activity 60 Ci/mmol; radiochemical purity >97.5%) was purchased from American Radiolabeled Chemicals (St. Louis, MO). All other cell culture reagents, reconstituted aqueous LPS (Escherichia coli 0111:B4) solution, and specific primers used for PCR amplifications were from Sigma Genosys (Woodlands, TX).

**Methods**

“Culture and transfection of the human epithelial cells. Confluent monolayers of the colonic epithelial NCM460 cells (derived from a 68-yr-old male), the intestinal epithelial Caco-2 cells (derived from a 72-yr-old male), and the retinal pigment epithelial ARPE-19 cells (derived from a 19-yr-old male) were used in these investigations (ATCC, Manassas, VA). NCM460 cells were maintained in F12 medium (Ham), whereas Caco-2 and ARPE-19 cells were maintained in EMEM (GIBCO, Waltham, MA) medium, supplemented with FBS (10%) and streptomycin (10 mg/l), under standard conditions. Confluent cell monolayers (3–4 days postconfluence) were used to examine the effect of LPS on biotin uptake. Cells were serum starved overnight, then treated with 50 µg/ml LPS in the appropriate growth medium supplemented with 0.5% FBS. ARPE-19 cells were used because of their proven high transfection efficiency of wild-type and mutant hSMVT constructs (43). One day before transfection, cells were seeded to attain 75–85% confluence at the time of transfection.

**Biotin uptake.** In the in vitro LPS exposure studies, confluent monolayers of NCM460 (as well as Caco-2 and ARPE-19 cells) were used, and biotin uptake was examined as described by us previously (16). In brief, cells were incubated (5 min; initial rate) in Krebs-Ringer (KR) buffer (133.00 mM NaCl, 4.93 mM KCl, 1.23 mM MgSO₄, 0.85 mM CaCl₂, 5.00 mM glucose, 5.00 mM glutamine, 10.00 mM HEPES, and 10.00 mM MES, pH 7.4) at 37°C in presence of [^3]H-biotin (6.4 nM). At the end of incubation, buffer was aspirated, and cells were washed twice with ice-cold KR buffer, lysed with 1 N NaOH (followed by neutralization with 10 N HCl) then counted for radioactivity in a liquid scintillation counter (Beckman Coulter, Brea, CA). Biotin uptake by the carrier-mediated process was determined by subtracting uptake of a physiological concentration of [^3]H-biotin in the presence of 1 mM unlabeled biotin from total uptake (i.e., from uptake in the absence of unlabeled biotin).

For in vivo studies, we used 12-wk-old male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME). The animal protocol was approved by the Long Beach VAMC Animal Care and Use Committee. Full-thickness proximal colonic sheets, prepared as described previously (17, 53), were used to examine the effect of in vivo exposure to LPS on colonic biotin uptake physiology and molecular biology parameters. In these experiments, colonic tissue was removed immediately after killing the animals, and equal pieces (1 cm) of colonic sheets were incubated (5 min) in KR (pH 7.4) buffer in the presence of [^3]H-biotin (64 nM), washed, and then processed for [^3]H content.

**CK2 activity assay.** NCM460 cells were homogenized in cell lysis buffer (Cell Signaling Technology, Danvers, MA). The phosphotransferase activity of CK2 was measured using a CK2 assay kit (Millipore, Billerica, MA) following manufacturer’s recommenda-
HC11002

The pcDNA 3.1 construct was used as a template, and PCR was performed using specific mutant primers (Table 1). The hSMVT-Santa Clara, CA) was used for mutating putative CK2 phosphorylation residues. The relative expression of different mRNAs was normalized relative to β-actin as an internal control and quantified by 2^-ΔΔCt method (34).

**Fig. 2.** Effect of LPS on the level of expression of total cellular sodium-dependent multivitamin transporter (SMVT) protein and mRNA in human colonic epithelial NCM460 cells (A) and mouse native colonic tissue (B). NCM460 cells were pretreated with LPS (50 μg/ml; 72 h) followed by determination of the level of expression of hSMVT protein (A, i) and mRNA (A, ii). Total cellular mSMVT protein (B, i) and mRNA level (B, ii) were determined in colonic tissue of LPS-treated mice (5 mg/kg body wt ip; 72 h). In both Western blot analyses, identical amounts of protein (60 μg) were loaded onto gels. Data were normalized relative to β-actin for mRNA and protein (see Methods). Data are means ± SE of at least 3 independent experiments. NS, not significant.

**Fig. 3.** Effect of LPS on expression of the hSMVT protein at the surface of NCM460 cells. Biotinylation assay was performed on LPS-treated (50 μg/ml; 72 h) and untreated cells. Inset: representative images from biotinylation assay. In the Western blot analysis, identical amounts of protein (60 μg) were loaded onto gels. Data are means ± SE of at least 3 independent experiments (*P < 0.01).
Western blot analysis. For Western blotting, cultured cells and mouse colonic tissues were homogenized in RIPA buffer (Sigma) in the presence of a complete protease inhibitor cocktail (Roche, Nutley, NJ). The soluble fraction was isolated by centrifugation at 8,000 g for 10 min, and the protein content was measured using DC protein assay kit (Bio-Rad). An equal amount of protein (60 μg) was loaded onto a 10% mini gel (Invitrogen) and transferred to a polyvinylidene fluoride (PVDF) membrane for Western blot analysis. The membrane was blocked overnight and then probed with specific anti-SMVT polyclonal antibodies (1:200 dilutions; Santa Cruz Biotechnology, Santa Cruz, CA); specificity of the antibodies has been previously validated in our laboratory using protein samples from the intestine of different species. Isolation of surface proteins was achieved by biotinylation using sulfo-NHS-SS-biotin (4°C; 30 min; Pierce Biotechnology) followed by isolation of surface proteins by incubating the cell lysate with streptavidin-agarose beads following the manufacturer’s protocol. Protein expression was validated with 1:25,000 dilutions for 1 h at room temperature described previously (18). Relative expression of the protein was quantified by normalizing the intensities with respect to corresponding β-actin using Odyssey application software (version 3.0) in an Odyssey Infrared imaging system (LI-COR).

Cell surface biotinylation assay. The effect of LPS, CK2 inhibitors, and mutating the putative CK2 phosphorylation sites in the SMVT protein on expression of the carrier protein at the cell membrane of NCM460 and ARPE-19 cells was examined by means of biotinylation assay using a cell surface biotinylation kit (Pierce Biotechnology, Rockford, IL), as previously described (6, 21, 33, 39). Briefly, cells were maintained under serum-starved condition overnight and treated with LPS (50 μg/ml; 72 h) in suitable medium supplemented with 0.5% FBS. For in vitro CK2 inhibition studies, cells were treated with CK2 inhibitors in serum-free medium (30 μM, 1 h). Cell surface biotinylation was performed by treating cells with sulfo-NHS-SS-biotin (4°C; 30 min; Pierce Biotechnology) followed by electrophoresis, the proteins were electro-blotted onto a PVDF immunoblot membrane.

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**Fig. 4.** Schematic diagram showing the putative CK2 phosphorylation site in the hSMVT protein. A: the hSMVT protein is predicted (by NetPhos 3.1 Server (9)) to have five putative CK2 phosphorylation sites (thick circles) that are conserved in different species. B: conserved amino acid residues (in rectangles) in different species were identified by multiple sequence alignment using “PRALINE” software (www.ibi.vu.nl/programs/pralinewww/).
and mRNA levels. Total cellular SMVT protein level was determined by means of Western blotting using whole cell homogenate and specific anti-SMVT polyclonal antibodies, whereas level of the SMVT mRNA was determined by realtime PCR (see Methods). The results showed no significant changes in the level of expression of total cellular hSMVT protein (Fig. 2Ai) or mRNA (Fig. 2Aii) in cells treated with LPS compared with untreated controls. Similarly, in vivo treatment of mice with LPS did not affect the level of expression of total cellular mSMVT protein or mRNA in the colonic mucosa compared with their levels in the colonic mucosa of the untreated controls (Fig. 2B, i and ii). These results suggest that the effect of LPS on colonic biotin uptake is not mediated via changes in the rate of transcription of the SLC5A6 gene or in total cellular level of the SMVT protein.

Effect of LPS on Biotin Uptake by Colonic Epithelial Cells

In this study, we examined the effect of treating (for 72 h) cultured human colonic epithelial NCM460 monolayers with the LPS (50 µg/ml, a concentration that is relevant to the endotoxin level that can be reached in colonic lumen) (24, 32, 50) on the initial rate (5 min) of carrier-mediated ³H-biotin (6.4 nM) uptake. The results showed a significant (P < 0.01) inhibition in the vitamin uptake by LPS-treated cells compared with uptake by simultaneously examined untreated controls (Fig. 1A). Similar results were obtained in studies utilizing the human intestinal epithelial Caco-2 cells, where again LPS (50 µg/ml; 72 h) caused a significant (P < 0.01) inhibition in carrier-mediated biotin uptake (Fig. 1B).

To establish physiological relevance of the above in vitro LPS exposure study, we also examined the effect of in vivo exposure of mice to LPS on biotin uptake by native colonic tissue. For this, adult mice were given a single dose of LPS (5 mg/kg body wt, i.p.) as described before (22), followed by examination (72 h later) of carrier-mediated biotin uptake by native colonic sheets as described by us before (17, 53). The results showed that in vivo exposure to LPS again leads to a significant (P < 0.01) inhibition in carrier-mediated biotin uptake by native colonic tissue (Fig. 1C).

Effect of In Vitro and In Vivo Exposure to LPS on Colonic Level of Expression of SMVT Protein and mRNA

Recent investigations from our laboratory have established that the SMVT is the only transport system responsible for biotin uptake in the gut (18). Thus we examined whether the above-described inhibition in carrier-mediated biotin uptake by in vitro exposure of the colonic epithelial NCM460 cells to LPS is associated with changes in expression of SMVT protein and mRNA levels. Total cellular SMVT protein level was determined by means of Western blotting using whole cell homogenate and specific anti-SMVT polyclonal antibodies, whereas level of the SMVT mRNA was determined by real-time PCR (see Methods). The results showed no significant changes in the level of expression of total cellular hSMVT protein (Fig. 2Ai) or mRNA (Fig. 2Aii) in cells treated with LPS compared with untreated controls. Similarly, in vivo treatment of mice with LPS did not affect the level of expression of total cellular mSMVT protein or mRNA in the colonic mucosa compared with their levels in the colonic mucosa of the untreated controls (Fig. 2B, i and ii). These results suggest that the effect of LPS on colonic biotin uptake is not mediated via changes in the rate of transcription of the SLC5A6 gene or in total cellular level of the SMVT protein.

LPS Inhibition of Colonic Biotin Uptake Is Mediated Via Reduction in Cell Surface Expression of the hSMVT Protein

With the above-described findings suggesting that LPS-mediated inhibition in colonic biotin uptake is not exerted at the level of transcription of the SLC5A6 gene or via changes in level of total cellular SMVT protein, we tested the possibility that the effect is mediated via changes in the fraction of the SMVT protein that is expressed at the cell surface. This was done by means of biotinylation assay followed by Western blotting. The results indeed showed a significant (P < 0.01) reduction in the level of expression of the hSMVT protein at the cell surface in LPS-treated NCM460 cells compared with untreated control cells (Fig. 3). These findings suggest that LPS interferes with the level of expression of SMVT at the cell surface of the colonic epithelial cells.

Role for CK2 in Mediating the LPS Effect on Colonic Biotin Uptake and on Membrane of Expression of the hSMVT Protein

Recent studies have reported a role for CK2 (11, 13) and protein kinase C (PKC) (23, 40) in the process of cell surface expression of membrane transporters in a variety of cellular systems. Thus we considered possible involvement of these pathways in mediating the LPS effect on membrane expression of the hSMVT protein (and on biotin uptake). We focused on the potential role of CK2 because our previous investigations have shown that PKC does not affect the expression of hSMVT at the cell surface (20) and that the sequence of the SMVT protein appears to have a number of putative CK2 phosphor-
ylation sites (Thr78, Ser128, Ser242, Thr366, and Thr627; Fig. 4), which are conserved among species.

First we examined whether treatment of the colonic epithelial NCM460 cells with LPS affects the activity of CK2 (see Methods). The results showed that such a treatment indeed leads to a significant ($P < 0.05$) inhibition in CK2 activity (Fig. 5A). We then examined the effect of pretreating (for 1 h) the colonic epithelial NCM460 cells with the CK2-specific inhibitors TBB and TBCA (both at $30 \mu M$) (58) on carrier-mediated biotin (6.4 nM) uptake. The results showed a significant ($P < 0.01$ for TBB and $P < 0.05$ TBCA compared with untreated control) inhibition in biotin uptake by both of the CK2 inhibitors tested (Fig. 5B). In a related study, we also tested whether exposing the LPS-treated NCM460 cells with TBB could further augment the inhibitory effect caused by LPS alone on biotin uptake. The result indeed showed that this is to be the case, with uptake of $1048.36 \pm 19.99$, $671.20 \pm 86.02$, and $330.09 \pm 46.69$ fmol/mg protein/5 min for control, LPS-treated cells, and LPS plus TBB-treated cells, respectively.

In another investigation, we examined whether the inhibition in biotin uptake caused by TBB is mediated via a decrease in the level of expression of the SMVT protein at the cell surface. We used the biotinylation assay described in Methods for this purpose. The results showed a significant ($P < 0.01$) decrease in the level of expression of the hSMVT protein at the cell membrane (Fig. 6A), with no change in its level in the total cell homogenate (Fig. 6B).

Fig. 6. Effect of the CK2 inhibitor TBB on expression of the hSMVT protein at the surface of NCM460 cells. Cells (maintained in serum-free medium) were treated with the TBB (30 $\mu M$; 1 h), followed by biotinylation assay (see Methods). A: surface expression of hSMVT was quantified using anti-hSMVT polyclonal antibodies and was normalized relative to total cellular hSMVT level. B: total cellular hSMVT was quantified and then normalized relative to $\beta$-actin. Inset: representative images from biotinylation assay. In both Western blot analyses, identical amounts of protein (60 $\mu g$) were loaded onto gels. Data are means $\pm$ SE of at least 3 independent experiments ($^*P < 0.01$).

Fig. 7. Effect of LPS (A) and CK2 inhibitors (B) on biotin uptake by the human retinal pigment epithelial ARPE-19 cells. Postconfluency cells were incubated with LPS (50 $\mu g/ml$, 72 h) or with the CK2 inhibitors TBB and TBCA (30 $\mu M$; 1 h) followed by examination of biotin uptake (see Methods). C: effect of mutating the putative CK2 phosphorylation sites in the hSMVT protein on biotin uptake. Equal amounts of the wild-type and the mutated hSMVT constructs (3 $\mu g$/well, in 12-well culture plates) were transfected into ARPE-19 cells. Biotin uptake was then determined 48 h after transfection. Data are means $\pm$ SE from at least 3 independent experiments ($^*P < 0.01$).
To further investigate the potential involvement of CK2 in the inhibitory effect of LPS on biotin uptake by colonic epithelial NCM460 cells and on cell surface expression of the hSMVT protein, we investigated the effect of mutating (to alanine) the putative CK2 phosphorylation sites in the hSMVT protein (Thr78Ala, Ser128Ala, Ser242Ala, Thr366Ala, and Thr627Ala) on carrier-mediated biotin uptake and on membrane expression of SMVT. The individual mutation was introduced into the hSMVT protein by means of site-directed mutagenesis followed by expression of the different mutant into ARPE-19 cells. We chose the latter cell type for this study because of their excellent transfection efficiency (unlike the poor transfection efficiency of NCM460 cells) and because the previous findings have established their suitability in studies of SMVT function and regulation (20, 25, 26). In these investigations, we first showed that treatment of the ARPE-19 cells with LPS or with the CK2 inhibitors TBB and TBCA also leads to inhibition (P < 0.01 for all) in biotin uptake (Fig. 7, A and B, respectively). We then examined the ability of the different hSMVT mutants (Thr78Ala, Ser128Ala, Ser242Ala, Thr366Ala, and Thr627Ala; Fig. 6) to transport biotin following their expression in ARPE-19 cells. The results showed a significant (P < 0.01; when compared with the hSMVT-WT construct) inhibition in carrier-mediated biotin (6.4 nM) uptake by cells expressing the Thr78Ala mutant but not those expressing the wild-type hSMVT or mutants Ser128Ala, Ser242Ala, Thr366Ala, and Thr627Ala (Fig. 7C).

To determine whether the decrease in the ability of the Thr78Ala hSMVT mutant to transport biotin is due to a decrease in level of expression of the protein at the cell surface, we again resorted to biotinylation assay. In this study, we transfected the ARPE-19 cells with the mutant Thr78Ala or with wild-type hSMVT followed by biotinylation assay. The results showed a significant (P < 0.05) reduction in the level of expression of the Thr78Ala mutant at the cell surface compared with level of expression of the wild-type hSMVT protein (Fig. 8).

DISCUSSION

Emerging evidence has pointed to the involvement of biotin in critical metabolic activities and physiological functions beyond its classical role as a cofactor for enzymes like carboxylases. This includes an expanded role for this micronutrient in normal immune function and response (5, 7, 18, 28, 29, 45), in the regulation of cellular oxidative stress (35), in the maintenance of normal gut mucosal physiology and homeostasis (18, 45), in influencing the colonization/invasiveness of certain entero-pathogenic bacteria (57), and in influencing gut microbial community (54). Thus studies that aim at furthering our understanding of the factors that affect interfere with normal body homeostasis of biotin are of clear physiological and nutritional importance.

The intestinal tract plays a critical role in regulating and determining body level of biotin, as humans (mammals) cannot synthesize the vitamin endogenously and thus must obtain the essential micronutrient from exogenous sources. The intestine encounters two sources of biotin, a dietary source (absorbed in the small intestine) and a microbiota-generated source (absorbed in the large intestine). Studies from our laboratory and others over the past quarter century have delineated different aspects of the carrier-mediated biotin uptake process and how specific external/internal conditions and factors affect the event. It is well established now that the intestinal biotin absorption process is mediated exclusively by the SMVT system, which is exclusively expressed at the apical membrane domain of absorptive epithelia (52). Thus any interference with the function/expression of this uptake system would have the potential to affect the normal intestinal absorption process of the vitamin.

In the present investigation, our aim was to examine the effect of LPS on the intestinal biotin uptake process. We focused on the effect of this bacterial endotoxin on colonic biotin uptake because the large intestinal lumen contains high levels of LPS (24, 32, 50) as it harbors the largest level of bacteria in the intestinal tract. We employed in vitro (cultured colonic epithelial NCM460 cells) and in vivo (mice) LPS exposure approaches in our investigations. Results of both approaches showed that exposure to LPS leads to a significant inhibition in colonic carrier-mediated biotin uptake. This inhibition was not mediated via changes in the level of expression of total cellular SMVT protein or in the level of its mRNA; rather it appeared to be mediated via a decrease in the fraction of the hSMVT protein that is expressed at the surface of colonic epithelial cells as indicated by the results of biotinylation assay.

It has been reported that both the CK2 and the PKC-mediated pathways play a role in regulating membrane expression of different proteins in a variety of cell types. Thus we considered the possible role of these two pathways in mediating the effect of LPS on membrane expression of the hSMVT protein and on biotin uptake. We focused on the role of the CK2-mediated pathway in this study because our previous investigations have shown no role for PKC in membrane expression of the hSMVT system (20) and because the hSMVT

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**Fig. 8.** Effect of mutating Thr78 of the hSMVT protein on its level of expression at the surface of ARPE-19 cells. Cells were transiently transfected with equal amounts of wild-type or mutated hSMVT (Thr78Ala). Biotinylation assay was performed (48 h after transfection), and surface expression of the hSMVT protein was quantified by means of Western blotting (see Methods). Identical amounts of protein (60 μg) were loaded onto gels. Data are means ± SE of at least 3 independent experiments (*P < 0.01).
polypeptide appears to have a number of potential CK2 phosphorylation sites. Our investigations showed that LPS inhibits the activity of CK2 in colonic epithelial NCM460 cells. Also inhibiting CK2 with the use of specific pharmacological inhibitors led to significant inhibition in both biotin uptake and in the level of expression of the hSMVT protein at the cell membrane without affecting the level of expression of the transporter in total cell homogenate. These findings suggest a role for CK2 in mediating the inhibitory effect of LPS on biotin uptake and on membrane expression of the hSMVT protein.

To further confirm the possible role of CK2 in regulating biotin uptake and in expression of the hSMVT protein at the cell surface, we examined the effect of mutating (individually) the putative CK2 phosphorylation sites in the SMVT polypeptide on biotin uptake. The results showed that mutating the Thr site, but not the other putative sites (Ser128, Ser242, Thr306 and Thr627), led to a significant inhibition in biotin uptake. This inhibition was again found to be associated with a decrease in the level of expression of the hSMVT protein at the cell surface with no change in total cellular level for the hSMVT protein.

In summary, results of these investigations show that colonic biotin uptake is sensitive to the inhibitory effect of the bacterial endotoxic LPS and that such an effect is mediated via a decrease in cell surface expression of the hSMVT protein. Furthermore, the latter appears to be exerted via involvement of a CK2-mediated pathway.

REFERENCES


