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Tumor necrosis factor α stimulates taurine uptake and transporter gene expression in human intestinal Caco-2 cells

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Abstract The effect of cytokines on the taurine uptake by human intestinal epithelial Caco-2 cells was investigated. Among the various cytokines tested, tumor necrosis factor α (TNF-α) markedly increased the taurine uptake by Caco-2 cells, resulting in an increase in the intracellular taurine level. TNF-α did not induce up-regulation of the taurine uptake in hepatic HepG2, renal human embryo kidney 293, and macrophage-like THP-1 cells. The uptake of glycine, L-leucine, and L-glutamic acid by Caco-2 cells was not affected by TNF-α. A kinetic analysis of the taurine uptake by TNF-α-treated Caco-2 cells suggests that this up-regulation was associated with both an increase in the amount of the taurine transporter (TAUT) and an increase in its affinity. TNF-α-treated cells showed a higher mRNA level of the TAUT than did the control cells.

Key words: Transporter; Taurine; Caco-2; Tumor necrosis factor α; Cytokine; Intestinal epithelial cell

1. Introduction

Taurine (2-aminoethanesulfonic acid), one of the major intracellular β-amino acids in mammals, is believed to be essential, particularly for the growth of fetuses and newborns. Taurine is involved in a variety of biological phenomena such as osmoregulation, antioxidation, detoxification, neuronal modulation, cell proliferation and cell viability [1,2].

Taurine is known to be transported by a specific transporter, the taurine transporter (TAUT). TAUT has been cloned from the brain [3,4], kidney [5], thyroid [6], and placenta [7]. We have cloned human intestinal TAUT in a form almost identical to that in other organs and demonstrated its regulatory characteristics in intestinal epithelial Caco-2 cells such as hypertonicity-induced up-regulation [8] and taurine-induced down-regulation [9]. We have also found that the taurine uptake was inhibited by food-derived substances and identified the inhibitory substance in sesame seeds as lysophosphatidylcholine [10].

In addition to these regulatory factors, another factor could contribute to the regulation of taurine transport; cytokines are candidates for TAUT regulators, because there have been several reports on cytokine-induced regulation of amino acid transport. For example, the L-arginine uptake by vascular smooth muscle cells is regulated by tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β) and tumor growth factor β (TGF-β) [11,12]. Kohan et al. [13] have also reported that IL-1 increased the D-aspartate uptake by the mouse renal epithelium. A highly developed immune system is known to work in the intestinal tract, where T lymphocytes, macrophages, fibroblasts and intestinal epithelial cells secrete various cytokines in response to extracellular stimulation [14–17]. Under such pathogenic conditions as inflammatory bowel diseases (IBD), the cytokine profile may dramatically change [18]. It is therefore possible that intestinal epithelial cells are frequently exposed to unusually high concentrations of cytokines secreted by other immunocompetent cells. However, the regulatory properties of intestinal TAUT by cytokines have not previously been reported.

The regulation of taurine uptake by cytokines was therefore investigated in the present study, and we found that TNF-α markedly increased the taurine uptake by human intestinal Caco-2 cells. The characteristics and mechanism for this regulation were investigated, and its physiological significance is also discussed.

2. Materials and methods

2.1. Materials

The Caco-2 cell line was obtained from the American Type Culture Collection (Rockville, MD, USA), and Dulbecco's modified Eagle's medium (DMEM) was purchased from Sigma (St. Louis, MO, USA). Penicillin–streptomycin (10 000 U/ml and 10 mg/ml in 0.9% sodium chloride, respectively) and non-essential amino acids were purchased from Gibco (Gaithersburg, MD, USA). Fetal calf serum was purchased from Asahi Technoglass (Chiba, Japan). [1,2-^{3}H]Taurine (specific radioactivity 29 Ci/mmol), [2-^{3}H]glycine (25 Ci/mmol), L-[4,5-^{3}H]leucine (155 Ci/mmol), L-[G-^{3}H]glutamic acid (49 Ci/mmol) and [α-^{32}P]dCTP were all from Amersham (Little Chalfont, UK). Human TNF-α, IL-1β, interleukin 5 (IL-5), interleukin 8 (IL-8), interleukin 15 (IL-15), TGF-β, and interferon γ (IFN-γ) were all purchased from Pepro Tech., Inc. (Rocky Hill, NJ, USA). All the other chemicals used were of reagent grade.

2.2. Cell culture

Caco-2 cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 in air with a culture medium consisting of DMEM, 10% fetal calf serum, 1% non-essential amino acids, 2% glutamine, 200 U/ml of penicillin, 200 μg/ml of streptomycin and an appropriate amount of

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Abbreviations: DMEM, Dulbecco's modified Eagle's medium; IBD, inflammatory bowel diseases; IFN-γ, interferon γ; IL-1β, interleukin 1β; IL-5, interleukin 5; IL-6, interleukin 6; IL-8, interleukin 8; IL-15, interleukin 15; NFκB, nuclear factor κB; PBS, phosphate-buffered saline; TAUT, taurine transporter; TGF-β, tumor growth factor β; TNF-α, tumor necrosis factor α

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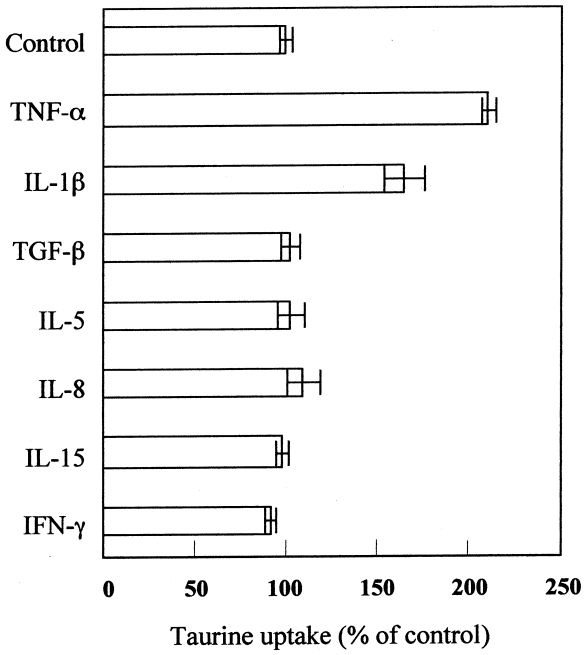

Fig. 1. Effect of various cytokines on the taurine uptake by Caco-2 monolayers. Cells were precultured for 24 h in a medium containing various TNF-α (50 ng/ml), TGF-β (50 ng/ml), IL-1β (50 ng/ml), IL-5 (50 ng/ml), IL-8 (50 ng/ml), IL-15 (50 ng/ml), or IFN-γ (1000 U/ml). Uptake experiments were then performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 4$).

sodium bicarbonate. HepG2, human embryo kidney 293 (HEK293), and THP-1 cells were cultured in the same medium as that for Caco-2, except that the non-essential amino acids were excluded. These cells were cultured at a density of 1.4×10^5 cells/well in 24-well plates that had been precoated with collagen and then used for the uptake experiments. The Caco-2 monolayers for the uptake experiments were used after 14 days of culture, the HepG2 cells after 5 days of culture, and the HEK293 cells after reaching 70–80% confluence. The THP-1 cells were differentiated to adherent cells by treating with 200 μM phorbol 12-myristate 13-acetate for 2 days before being used for the uptake experiment.

2.3. Uptake experiments

[^{3}H]Taurine uptake experiments were performed in the absence (total uptake) or presence (non-specific uptake) of 50 mM unlabeled taurine, thus allowing the specific uptake to be calculated by subtraction. The cells were washed twice with 700 μl each of Hank's balanced salt solution containing 4 mM sodium bicarbonate and 10 mM HEPES, the pH value being adjusted to 7.4 with potassium hydroxide (uptake buffer). The cells were then incubated at 37°C for 10 min with 0.3 μCi of [^{3}H]taurine in 300 μl of the uptake buffer, with or without 50 mM taurine. At the end of the incubation period, the buffer was removed, and each monolayer was carefully washed three times with 700 μl each of ice-cold phosphate-buffered saline (PBS) containing 0.05% sodium azide for 5 min. To each well was then added 250 μl of 0.1% Triton X-100, before the dissolved cells were 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).

2.4. Measurement of the intracellular content of amino acids

To measure the intracellular content of amino acids, Caco-2 cells cultured in six-well plates for 14 days were used. The cells were rinsed twice with PBS and rendered soluble with 0.1% Triton X-100. The resulting cell homogenate was mixed with an equal volume of 10% trichloroacetic acid and centrifuged at 10 000 × g for 10 min. The amino acid content of the supernatant was measured with an L-8500 high-speed amino acid analyzer (Hitachi, Japan).

2.5. Northern blot analysis

Poly(A)$^+$ RNA (10 μg) that had been fractionated on 1% agarose gel containing 2.2 M formaldehyde was transferred to a nylon filter (Hybond-N, Amersham) according to the manufacturer's instructions. The filter was hybridized with the polymerase chain reaction product of the human TAUT [7] that had been labeled by randomly priming with an [α-^{32}P]dCTP labeling kit (Multiprime, Amersham). After treating in a hybridization solution (Rapid, Amersham) at 65°C for 3 h, the filter was washed in 0.1 × SSC containing 0.1% sodium dodecyl sulfate at 65°C.

3. Results

3.1. Effect of cytokines on the taurine uptake by Caco-2 cells

Caco-2 cells after 14 days of culture were incubated with the various cytokines for 24 h each, and uptake experiments were then performed. Among the cytokines tested, TNF-α and IL-1β, respectively, increased the taurine uptake activity by 200% and 160% of the control value (Fig. 1). The time-

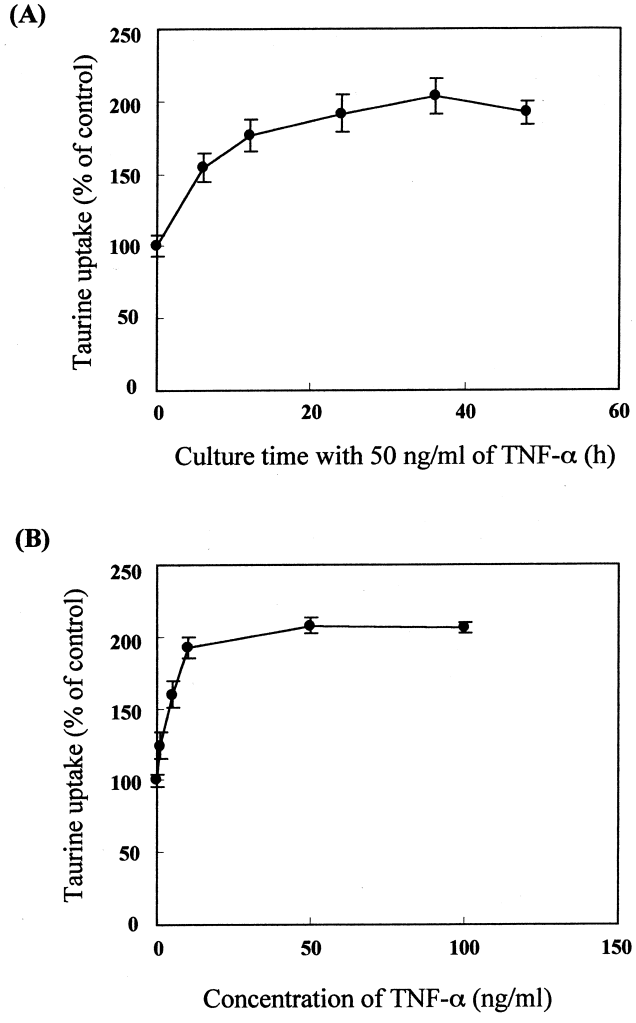

Fig. 2. Time- and concentration-dependence of the TNF-α-induced up-regulation of taurine uptake in Caco-2 monolayers. A: Caco-2 cells were precultured in a medium containing TNF-α (50 ng/ml) for various times (0–48 h), and uptake experiments were performed as described in Section 2. Each value is the mean ± S.E.M. ($n = 4$). B: Cells were precultured for 24 h in a medium containing various concentrations of TNF-α. Uptake experiments were then similarly performed. Each value is the mean ± S.E.M. ($n = 4$).

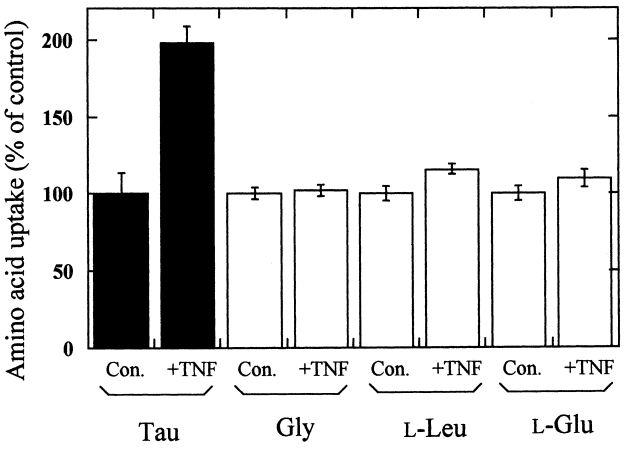

Fig. 3. Effect of TNF-α on the uptake of various amino acids by Caco-2 cells. Cells were precultured for 24 h in a medium with 50 ng/ml of TNF-α (+TNF) or without (Con.). Uptake experiments were then similarly performed. Each value is the mean ± S.E.M. ($n = 4$).

and concentration-dependence of TNF-α-induced up-regulation of the taurine uptake by Caco-2 cells was then examined. The cells were incubated with 50 ng/ml of TNF-α for 0–48 h, and the taurine uptake activity was measured. The taurine uptake increased in a time-dependent manner from 0 to 24 h and reached a plateau after 24 h (Fig. 2A). The cells were also incubated with different concentrations of TNF-α (1–100 ng/ml) for 24 h before the uptake experiments. As shown in Fig. 2B, the taurine uptake increased as the concentration of TNF-α exposed to Caco-2 increased. The activity reached nearly 200% of the control value when the cells were exposed to a 10 ng/ml or higher concentration of TNF-α.

3.2. Effect of TNF-α on the uptake and cellular accumulation of other amino acids

We investigated the effect of TNF-α on the uptake of glycine (Gly), L-leucine (L-Leu), and L-glutamic acid (L-Glu) by Caco-2 cells. As shown in Fig. 3, TNF-α had no effect on the Gly, L-Leu, or L-Glu uptake, whereas the taurine uptake was markedly increased.

The intracellular amino acid content in Caco-2 cells cultured with TNF-α was next measured. Among the various amino acids, only the taurine content significantly increased in TNF-α-treated cells, reaching 130% of the control value (data not shown). This increase was dependent on the culture time with TNF-α (data not shown).

3.3. Effect of TNF-α on the taurine uptake by HepG2, HEK293, and THP-1 cells

The TAUT is expressed not only in the intestines but also in other tissues. We therefore investigated whether the taurine uptake by human hepatoma HepG2, HEK293, and human macrophage-like (THP-1) cells was also up-regulated by TNF-α. However, little or no change was observed in the taurine uptake by these cells (Fig. 4), suggesting that the TNF-α-induced up-regulation of taurine uptake was tissue specific.

3.4. Kinetics of the taurine uptake by Caco-2 cells cultured with TNF-α

A kinetic analysis of the taurine uptake was performed on cells cultured with or without 50 ng/ml of TNF-α for 24 h. The V_{max} value for the control cells was 417 pmol/mg of protein/10 min, and the K_m value was 17.3 μM. For the TNF-α-treated cells, the V_{max} and K_m values were 769 pmol/mg of protein/10 min and 11.9 μM, respectively (Table 1). These results indicate that the TNF-α-induced up-regulation of the taurine uptake was mainly due to an increase in the number of cell-surface TAUTs, although a change in the affinity of the TAUT was also likely to have been involved in this regulation.

3.5. Expression level of TAUT mRNA in Caco-2 cells cultured with TNF-α

The Northern blot analysis was performed to determine whether or not this phenomenon was associated with a change in the expression level of TAUT mRNA. Poly(A)$^+$ RNA (10 μg) extracted from Caco-2 cells that had been cultured in a medium with 50 ng/ml of TNF-α for 0–24 h was used. Fig. 5 shows that the expression level of TAUT mRNA was markedly increased by the TNF-α treatment in a time-dependent manner. This suggests that the up-regulation of taurine uptake by TNF-α was attributable to the increased expression level of TAUT mRNA.

4. Discussion

We investigated in the present study, using human intestinal Caco-2 cells, whether cytokines regulated the taurine uptake by the intestinal epithelial cells. The results show that TNF-α markedly increased the taurine uptake by Caco-2 cells. The up-regulation by TNF-α was taurine specific and also tissue specific, and is likely to have been due to an increase in the maximal velocity of the TAUT, as well as due to a change in the affinity. The TNF-α-induced increase in the expression level of TAUT mRNA would account for this increase in the V_{max} value, although the cellular events in the TNF-α-induced up-regulation of TAUT have not yet been revealed.

Since TNF-α is known to activate nuclear factor κB (NFκB), one of the important transcriptional factors [19], activation of the NFκB pathway may be involved in this regulation. Our preliminary experiment has demonstrated that the TNF-α-induced increase in the taurine uptake was diminished by treating the cells with specific inhibitors of the

Table 1
Kinetic analysis of the taurine uptake by TNF-α-treated and control cells

	Control	TNF-α-treated
V_{max} (pmol/mg of protein/10 min)	417	769
K_m (μM)	17.3	11.9

Cells were precultured in a medium containing 50 ng/ml of TNF-α for 24 h. The taurine uptake by the TNF-α-treated and control cells was then measured over the concentration range of 1–50 μM taurine.

NFκB pathway such as pyrrolidinedithiocarbamate, thus supporting this hypothesis. Cloning and characterization of the promoter region of the human intestinal TAUT gene are now in progress to obtain more direct evidence for the participation of NFκB in the regulation of TAUT gene transcription.

TNF-α had little or no effect on the taurine uptake by human hepatic, renal and immune cell lines as shown in Fig. 3, suggesting that this phenomenon was specific to the intestinal epithelial cells. During this study, however, Chang et al.[20] have reported that the taurine uptake by rat astrocytes was increased by TNF-α, although the behavior or regulation of the TAUT in this phenomenon has not been analyzed in their paper. This finding, however, indicates that the TNF-α-induced up-regulation of TAUT is not always restricted to the intestinal epithelial cell, and that nerve cells also possess a similar mechanism. This may suggest that taurine plays a similar or common physiological role in these two types of cells.

The physiological significance of the up-regulation of TAUT is most interesting. It is known that an inflammatory condition called 'controlled inflammation' [21] takes place in the intestines due to cytokine-mediated interaction between lamina propria lymphocytes and epithelial cells, although the intestinal inflammatory reactions are usually balanced and regulated by various cytokines [22]. TNF-α plays a key role in the development of inflammation. TNF-α, as well as IL-1 and interleukin 6 (IL-6), increase the ability of epithelial cells, endothelial cells, macrophages and B cells to secrete potent chemokines such as IL-8 and monocyte chemotactic protein-1, by which macrophages and granulocytes are moved to the inflamed mucosa. Isaacs et al. [23] have shown a more frequent occurrence of TNF-α, IL-1 and IL-6 in IBD patients than in normal patients. Several clinical studies have also demonstrated that anti-TNF-α therapy reduced the symptoms in IBD patients [24]. These findings suggest the importance of TNF-α in the development of inflammation.

The intestinal inflammation induced by TNF-α is accompanied by disruption of the intestinal epithelial barrier functions [25]. Apoptosis in the epithelial cells induced by TNF-α would be one of the reasons of this disruption. Interestingly, TNF-α also negatively regulates apoptosis by activating NFκB. Soler et al. [26] have reported that disruption of the epithelial barrier function caused by TNF-α was self-recovered and that the restoration of the barrier was due to the activation of NFκB. Our experimental results suggest that the NFκB activation was linked to the up-regulation of TAUT. Taken together, the TNF-α-induced increase in the intracellular taurine concentration may play a role in the NFκB-induced restoration of the epithelial barrier function, because taurine is an intracellular antioxidant and also an anti-inflammatory agent with anti-apoptotic activity [27]. The up-regulation of TAUT could therefore be a reasonable response for intestinal epithelial cells under inflammatory conditions.

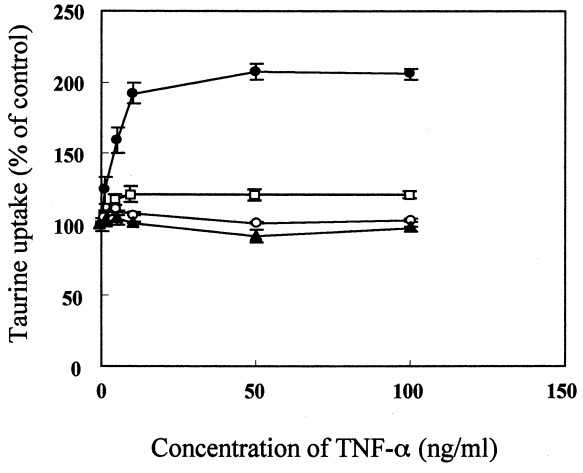

Fig. 4. Effect of the TNF-α treatment on the taurine uptake by Caco-2, HepG2, HEK293, and THP-1 cells. Four cell lines (Caco-2 (●), HepG2 (○), HEK293 (▲) and THP-1 (□)) were each precultured for 24 h in a medium containing various concentrations of TNF-α. Uptake experiments were then similarly performed. Each value is the mean ± S.E.M. ($n = 4$).

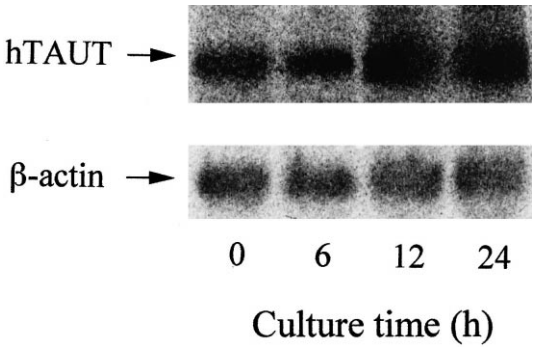

Fig. 5. Northern blot analysis of TAUT mRNA from Caco-2 cells cultured with TNF-α. 10 μg of poly(A)$^+$ RNA isolated from Caco-2 cells cultured with 50 ng/ml of TNF-α for different times (0, 6, 12, 24 h) was subjected to a Northern blot analysis. β-Actin cDNA was used as the control.

It has been reported that proinflammatory cytokines such as TNF-α and IL-8 were expressed in intestinal epithelial cells in response to bacterial invasion and were up-regulated after TNF-α stimulation [28]. Seabra et al. [29] have reported that taurine blunted the lipopolysaccharide-induced increase in TNF-α by Kupffer cells. If taurine suppresses the excess production of such inflammatory cytokines as TNF-α in the intestinal epithelial cells as well, the up-regulation of TAUT by TNF-α would also be a reasonable response.

In conclusion, we found for the first time that the human intestinal TAUT was up-regulated by TNF-α and that the expression of TAUT mRNA was also up-regulated. Studying the regulatory mechanism and its physiological significance may lead to finding new functions of taurine.

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