약제학회지(2008), 제 38 권 제 5 호
J. Kor. Pharm. Sci., Vol. 38, No. 5, 295-302 (2008)

The Regulation of Taurine Transport through the Blood-Placental Barrier under Oxidative Stress

Young Sook Kang† and Yoo Geum Yoon

College of Pharmacy, Sookmyung Women's University, Seoul, Korea

(Received August 10, 2008 · Revised September 12, 2008 · Accepted September 25, 2008)

ABSTRACT – In the present study, we examined the changes of uptake and efflux of taurine under various conditions inducing oxidative stress using rat conditionally immortalized syncytiotrophoblast cell line, TR-TBT cell, as blood-placental barrier *in vitro* model. In addition, we identified the characteristics of taurine transport in TR-TBT cells including general features, besides effect of calcium ion on taurine transport. Taurine uptake showed time, Na^+ and Cl^- dependency, and was decreased by PKC activator in TR-TBT cells. Also, calcium free condition decreased taurine uptake and evoked taurine efflux in the cells. Oxidative stress induced the change of taurine transport in TR-TBT cells, but the changes were different depending on the types of stimulation inducing oxidative stress. The taurine uptake was increased by TNF-α, LPS and DEM stimulation but decreased by H_2O_2 and NO stimulation. Also, the taurine efflux was regulated by TNF-α stimulation. In conclusion, the taurine transport through the blood-placental barrier was regulated in oxidative stress conditions, and these results demonstrated that oxidative stress affected the taurine supplies to fetus and taurine level of fetus.

Key words – Blood-placental barrier, TR-TBT, Taurine transporter, Oxidative stress, Calcium ion

Taurine exists in high concentration in most tissues, where it has various physiological functions, including conjugation with bile acids, anti-oxidation, detoxification, osmoregulation, membrane stabilization and modulation of intracellular calcium level.[1-4] Taurine can be supplied by diet or biosynthesis in human body. However, being absent or lacking in the fetus due to deficiency of enzyme, cysteine sulfinate decarboxylase,[2] taurine is an essential amino acid at fetal and neonatal stages. Thus, transport of taurine through the placenta is the only route for taurine supply from mother to fetus. Taurine plays an important role in fetal development, and it has been reported that taurine deficiency during pregnancy is associated with growth retardation, retinal degeneration and dysfunction of the CNS.[5,6]

Nutrients transport into fetus is acting through syncytiotrophoblast which is the layer of placenta villi between maternal blood and fetal blood.[7] Human placental syncytiotrophoblast possesses an active high-affinity transport system for taurine and the taurine transporter (TauT) is a main transporter involved in taurine transport.[8,9] TauT is dependent on sodium and chloride ion and regulated by PKC, glucose, hypertonicity and cytokine in various cells and organs, such as brain, retina, intestinal cell, hepatic cell and placenta.[2]

We focused on the effect of oxidative stress on taurine transport in placenta, because taurine plays a role as antioxidant in the cells and oxidative stress affects taurine supplement into fetus. Oxidative stress in the cells is induced by increased oxidant generation, decreasing oxidant protection and failure of repairing oxidative damage. A reactive oxygen species (ROS) is generated *in vivo* by aerobic respiration, metabolism of xenobiotic compounds and inflammation induced by phagocytosis process.[10] In the recent report, it showed that the level of taurine in the fetus at intrauterine growth restriction (IUGR) was low,[11] NO level was also high in IUGR pregnancies.[12] It suggests that a mutual relation between oxidative stress and taurine transport. Thus, it is necessary to investigate the effect of oxidative stress on taurine transport in placenta.

In the present study, we examined taurine transport under various conditions inducing oxidative stress, such as pro-inflammatory cytokine, tumor necrosis factor-α; bacterial endotoxin, lipopolysaccharide; compound inducing a depletion of antioxidant such as glutathione, diethyl maleate; ROS compound, hydrogen peroxide; nitric oxide donor. Many reports have demonstrated taurine uptake only, but in this study, taurine efflux was studied as well as taurine uptake under oxidative stress.

Kitano *et al.* established a conditionally immortalized rat syncytiotrophoblast cell line, TR-TBT 18d-1 and 18d-2 cells, from pregnant Tg-rat placenta at gestational day 18.[13] TR-TBT 18d-1 and 18d-2 were originated from the syncytiotrophoblast I (maternal side) and the syncytiotrophoblast II (fetal

†본 논문에 관한 문의는 이 저자에게로
Tel : 02)710-9562, E-mail : yskang@sookmyung.ac.kr

side), respectively, because rat syncytiotrophoblast consists of two layers while that of human is composed with one layer. Kitano *et al.* showed that TR-TBT is a good model for the analysis of the placental transport of nutrients.[13,14] We have carried this study using the blood-placental barrier *in vitro* model, TR-TBT, and identified not only the general characteristics of taurine transport in TR-TBT cells, but also the effect of calcium ion in taurine transport.

Materials and Methods

Materials

Radiolabeled [^{3}H]taurine (SA 20.1 Ci/mmol) was obtained from NEN Life Science Products Inc.(Boston, MA, USA). Tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS), 3-morpholinosyndomine (SIN-1), phorbol 12-myristate 13-acetate (PMA), chelerythrine, taurine, β-alanine, γ-amino butyric acid (GABA), nifedipine, nimodipine, verapamil, nickel chloride and cadmium chloride were purchased from Sigma Chemical (St. Louis, MO, USA). Diethyl maleate (DEM) and hydrogen peroxide (H_2O_2) was obtained from Aldrich chemical Co. and Junsei chemical Co., respectively.

Cell culture

The TR-TBT cells were cultured with Dulbelcco's modefied Eagle's medium (DMEM; invitrogen, Grand Island, NY), supplemented with 10% fetal bovine serum (FBS; Invitrogen), 100 U/mL penicillin (PC), and 100 μg/mL streptomycin (SM; both from invitrogen) at 33°C in a humidified atmosphere of 5% CO_2/air.

Uptake studies

TR-TBT cells (1×10^5 cells/well) were cultured on collagen type I coated 24 well plates (Iwaki Co.) at 33°C for 3 days and then at 37°C for 3 days. After washing the cells three times with ECF buffer (122 mM NaCl, 25 mM $NaHCO_3$, 10 mM D-Glucose, 3 mM KCl, 1.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.4 mM K_2HPO_4 and 10 mM HEPES), 200 μL ECF buffer containing [^{3}H]taurine (28 nM) was added to the cells for desired time. Under Ca^{++} free condition, Ca^{++} was replaced with Mg^{++}. [^{3}H]Taurine uptake was stopped by removing the isotope ECF buffer and washing the cells with ice ECF buffer three times. To investigate the change of taurine uptake under the oxidative stress condition, the TR-TBT cells were pretreated with 20 ng/mL TNF-α, 10 ng/mL LPS, 100 μM DEM, 100 μM H_2O_2, 2 mM H_2O_2 or 1 mM SIN-1 for 3, 6, 9, 12 and 24 h and the uptake study was performed as described above. The cells were solubilized in 750 μL 1N NaOH overnight. The solution (500 μL) was mixed with scintillation cocktail (Packard Co., Meriden, CT) for measurement of radioactivity in a liquid scintillation counter (LSC 6500, Beckman, Fullerton, CA). Cellular protein was quantified using a protein assay kit (Bio-Rad Laboratories Co., Hercules, CA, USA).

Efflux studies

After cell culture, the same as uptake experiment, ECF buffer containing [^{3}H]taurine was added to the cells for 60 min. The isotope buffer was removed and washed three times with ice buffer and then added 37°C ECF buffer 1 mL to the cells for the desired time. After incubation for efflux, ECF buffer was removed and the amount of [^{3}H]taurine remaining in the cells was measured. To induce oxidative stress in the cells, the TR-TBT cells were pretreated with 20 ng/mL TNF-α, 10 ng/mL LPS and 100 μM DEM for 9 and 24 h and the efflux study was performed as described above.

Data analysis

All data are given as mean ± SEM values. An unpaired, two-tailed Student's t-test was used to determine the significance of differences between two group means and $p<0.05$ was considered statistically significant.

Results

Characteristics of the taurine transport by TR-TBT cells

To identify taurine transport in TR-TBT cells, we inves-

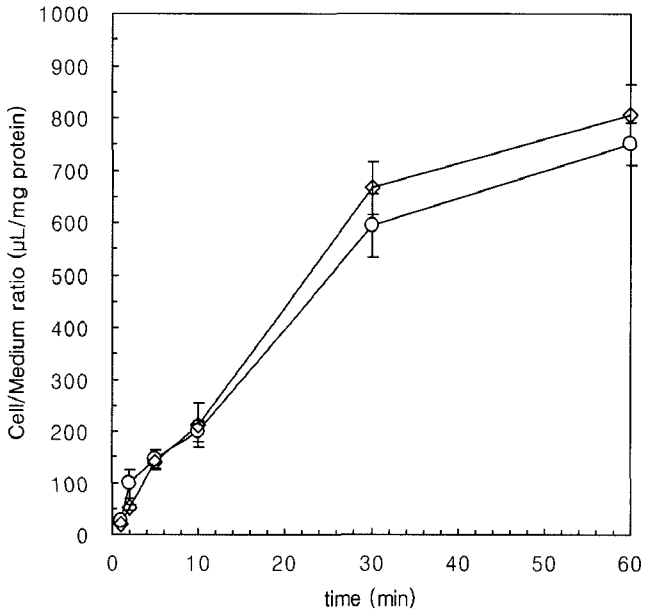

Figure 1—Time-course of [^{3}H]taurine uptake by TR-TBT 18d-1 (○) and TR-TBT 18d-2 (◇) cells. [^{3}H]Taurine (136.8 nM) uptake was performed at 37°C and measured at 1, 2, 5, 10, 30, 60 min. Each point represents the mean ± SEM (n=4).

tigated time and ion dependence of taurine transport. The taurine uptake was increased time-dependently and it was linear for 30 min (Fig. 1). Table I showed that the taurine uptake by TR-TBT cells was dependent on sodium and chloride ion concentration. It was inhibited over 90% under sodium and chloride ion free condition. Also, taurine transporter substrates such as taurine, β-alanine and γ-amino butyric acid (GABA) at a concentration of 1 mM inhibited the taurine uptake significantly 96%, 94% and 82% (18d-1) and 96%, 94% and 67% (18d-2), respectively (Table I). Furthermore, taurine transport was regulated by phosphorylation of protein kinase C (PKC) in TR-TBT cells. As exposed to PKC activator for 30 min, phorbol 12-myristate 13-acetate (PMA) 1 mM, taurine uptake was significantly decreased (Table II).

Effect of calcium ion and calcium channel blockers on the taurine uptake by TR-TBT cells

Taurine is a modulator of intracellular calcium homeostasis[4] and the effect of calcium ion on taurine transport was reported.[15] We investigated the effect of calcium ion on taurine uptake in TR-TBT cells by removal of calcium ion from extracellular buffer or addition of various calcium channel blockers. Calcium ion was replaced with magnesium ion and the taurine uptake represented 70% and 87% in TR-TBT 18d-1 and TR-TBT 18d-2 cells under calcium free condition compared with normal condition (Table III). 0.3 mM verapamil, 2 mM nifedipine, 2 mM nimodipine, 1 mM $NiCl_2$ and 0.3 mM $CdCl_2$, as calcium channel blockers, were added to extracellular buffer and these conditions induced a decrease of the taurine uptake except a nimodipine (Table III). However, at the concentration of two folds calcium ion, the taurine uptake had no change in TR-TBT 18d-1 cells, and increased slightly in TR-TBT 18d-2 cells.

Effect of oxidative stress, TNF-α, LPS and DEM, on the taurine uptake by TR-TBT cells

We investigated a change of the taurine uptake under oxidative stress conditions in TR-TBT cells. To induce oxidative stress conditions, the cells were incubated in tumor necrosis factor-α (TNF-α) 20 ng/mL as a cytokine, lipopolysaccharide (LPS) 10 ng/mL as a bacterial endotoxin, and diethyl maleate (DEM) 100 mM as compound inducing oxidative stress, for 24 h. The taurine uptake was increased, respectively, 139, 124 and 137% (18d-1) and 150, 128 and 154% (18d-2) (Fig. 2). Fig. 3 showed a change of taurine uptake as incubation time under exposure to compounds inducing oxidative stress, TNF-α, LPS and DEM. The taurine uptake was increased as incubation time and it showed maximal uptake at 9 or 12 h and it fell slightly after 24 h treatment compared with 9 or 12 h, except LPS treatment in TR-TBT 18d-2 cells, but the uptake amount was still larger than control without treatment.

Table I–*Inhibition of [^{3}H]taurine Uptake by Ion Dependency and TauT Substrates in TR-TBT Cells*

ECF condition	[^{3}H]Taurine uptake (% of control)	
	TR-TBT 18d-1	TR-TBT 18d-2
Control	100±7	100±1
Na^+ free	7.65±1.51*	3.50±0.29*
Cl^- free	10.1±1.1*	3.94±0.24*
taurine 1 mM	3.76±0.84*	4.30±1.10*
β-alanine 1 mM	6.13±0.97*	5.95±0.65*
GABA 1 mM	18.6±3.4*	33.0±0.65*

[^{3}H]Taurine (28 nM) uptake was performed at 37°C for 5 min in the absence of sodium and chloride ion or in the presence of taurine transporter substrates such as 1 mM taurine, β-alanine and γ-amino butyric acid. Sodium and chloride ion were replaced with choline or potassium and gluconate. Each value represents the mean±SEM (n=3-4). *p<0.001 significantly different from control.

Table II–*Inhibition of [^{3}H]taurine Uptake by PKC Activator in TR-TBT Cells*

PKC activator treatment (30 min)	[^{3}H]Taurine uptake (% of control)	
	TR-TBT 18d-1	TR-TBT 18d-2
Control	100±5	100±2
PMA 1 μM	82.7±2.6*	85.6±1.6**

[^{3}H]Taurine (28 nM) uptake was performed at 37°C for 5 min. After pretreatment of protein kinase C activator, phorbol 12-myristate 13-acetate (PMA), for 30 min, [^{3}H]taurine uptake was performed. Each value represents the mean ± SEM (n=3).
*p<0.05, **p<0.01 significantly different from control.

Table III–*Effect of Calcium Ion and Calcium Channel Blockers on [^{3}H]taurine Uptake by TR-TBT Cells*

ECF condition	[^{3}H]Taurine uptake (% of control)	
	TR-TBT 18d-1	TR-TBT 18d-2
Control	100±9	100±3
Ca^{++} free	70.4±6.1*	87.0±2.8*
Ca^{++} 2fold	96.6±14.6	122±8*
$NiCl_2$ 1 mM	50.7±3.5**	72.9±1.9**
$CdCl_2$ 2 mM	36.5±1.3**	90.5±2.0**
Verapamil 0.3 mM	15.1±2.7**	31.4±2.1**
Nifedipine 2 mM	80.7±1.0**	83.7±3.4**
Nimodipine 2 mM	105±1.0**	96.1±7.8

[^{3}H]Taurine (28 nM) uptake was performed at 37°C for 5 min. Under Ca^{++} free condition, Ca^{++} was replaced Mg^{++}. 1 mM nickel chloride, 0.3 mM cadmium chloride, 0.3 mM verapamil, 2 mM nifedipine and 2 mM nimodipine as calcium channel blockers were added to ECF buffer. Each value represents the mean ± SEM (n=3-4).
*p<0.05, **p<0.01 significantly different control.

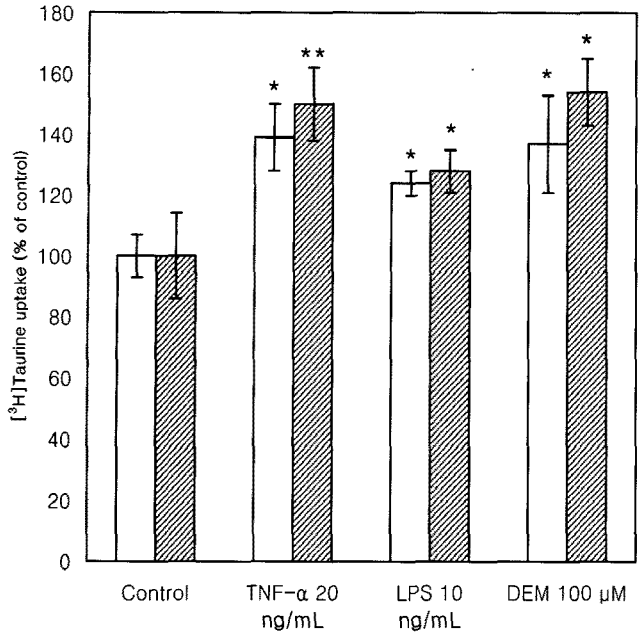

Figure 2–The uptake of [^{3}H]taurine by TR-TBT (18d-1 (□), 18d-2 (▨)) cells under oxidative stress for 24 h treatment. Cells were treated with tumor necrosis factor-α (TNF-α, 20 ng/mL), lipopolysaccharide (LPS, 10 ng/mL) and diethyl maleate (DEM, 100 μM) for 24 h, respectively. [^{3}H]Taurine uptake was performed at 37°C for 5 min. Each value represents the mean ± SEM (n=3-4). *$p<0.05$, **$p<0.01$ significantly different from the control.

Effect of oxidative stress, hydrogen peroxide and nitric oxide, on the taurine uptake by TR-TBT cells

As exposed to 2 mM H_2O_2 for 24 h, the taurine uptake was decreased about 85% and 80% in TR-TBT 18d-1 and 18d-2 cell (Fig. 4). In the lower concentration 100 mM H_2O_2, change was not shown. Also, exposure to nitric oxide donor, SIN-1, induced a decrease of taurine uptake significantly (Fig. 4). Incubation in 1 mM SIN-1 for 24 h induced reductions of taurine uptake, 80% (18d-1) and 84% (18d-2). At a concentration of 2.5 mM SIN-1 for 2 h, the taurine uptake was decreased 79% and 88% in TR-TBT 18d-1 and 18d-2 cells, respectively.

The taurine efflux in normal and various conditions by TR-TBT cells

Time-course of taurine efflux for 30 min was examined in TR-TBT cells. By measuring the taurine efflux from the cells during 0, 2, 5, 10, 20 and 30 min incubation after 60 min taurine uptake, the remaining taurine percentage in the cells was decreased time-dependently (Fig. 5). The taurine efflux rate was about 30% during 30 min incubation. Effect of TauT substrate on the taurine efflux was examined by addition of TauT substrates, 1 mM taurine, β-alanine and GABA, in the efflux ECF buffer. In the presence of taurine and β-alanine, the taurine efflux was inhibited significantly in both 18d-1 and 18d-2 cell compared with the normal taurine efflux, but GABA did not affect taurine efflux (Fig. 6). Under calcium depletion condition, the taurine efflux was evoked significantly (Fig. 7). Also, the taurine efflux was affected by exposure to TNF-α for 9 h, (Fig. 8) at the incubation time where maximal taurine uptake (Fig. 3) was represented. Figure 8 showed the time-course of taurine efflux for 30 min under exposure to oxidative stress for the designated time represented maximal taurine

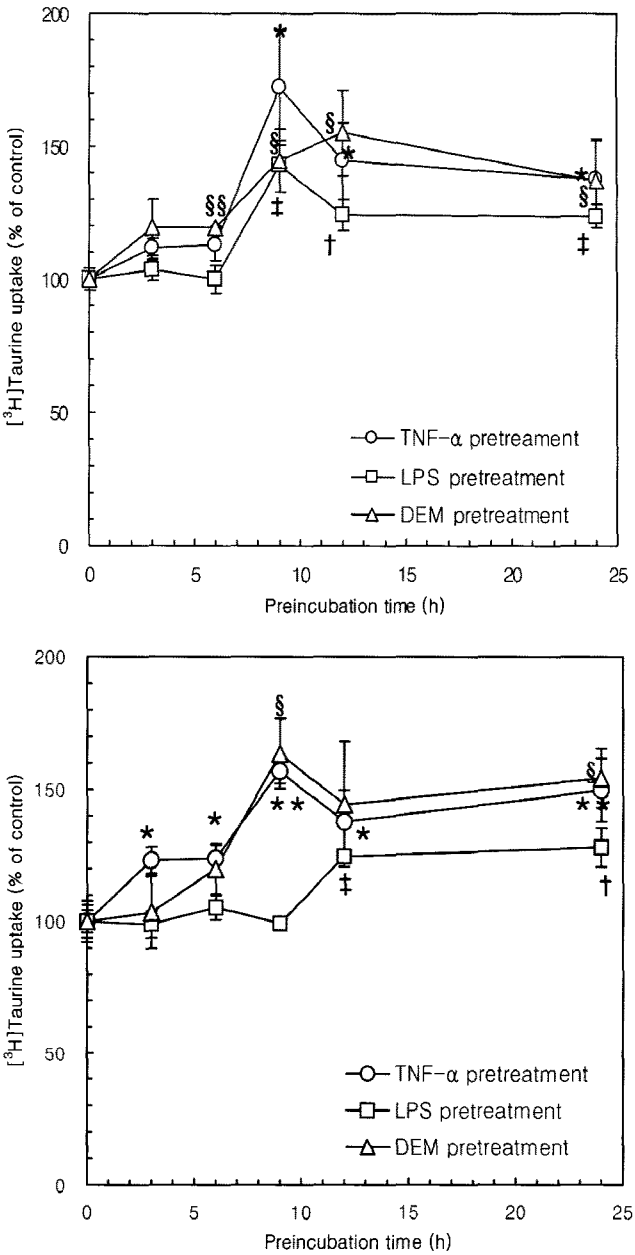

Figure 3–Time-course of [^{3}H]taurine uptake in oxidative stress condition at TR-TBT 18d-1 (upper), 18d-2 (lower) cells. Cells were treated with TNF-α (20 ng/mL), LPS (10 ng/mL) and DEM (100 μM) for 3, 6, 9, 12 and 24 h. [^{3}H]Taurine uptake was performed at 37°C for 5 min. Each value represents the mean ± SEM (n=3-4). *p<0.05, **p<0.01 (TNF-α), †p<0.05, ‡p<0.01 (LPS), §p<0.05, §§p<0.01 (DEM) significantly different from time=0.

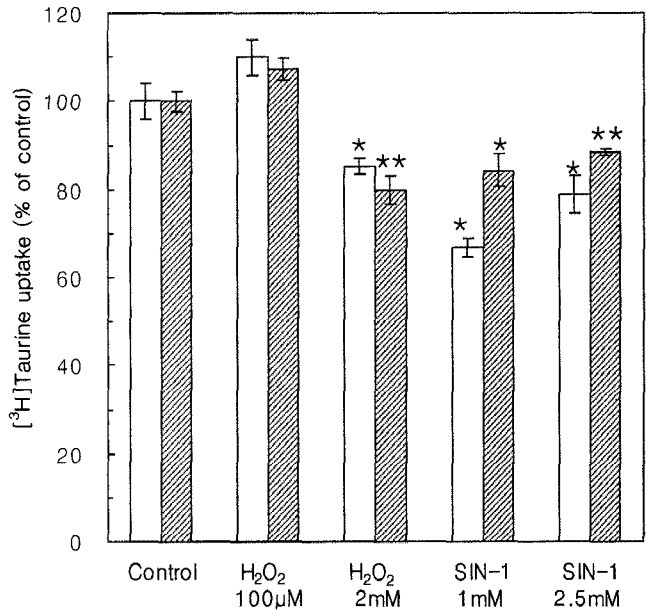

Figure 4–The uptake of [^{3}H]taurine by TR-TBT 18d-1 (□), 18d-2 (▨) cells under hydrogen peroxide and NO donor treatment. Hydrogen peroxide 100 μM and 2 mM for 24 h and SIN-1 1 mM for 24 h, SIN-1 2.5 mM for 2 h. [^{3}H]Taurine uptake was performed at 37°C for 5 min. Each value represents the mean ± SEM (n=3-4). *$p<0.05$, **$p<0.01$ significantly different from the control.

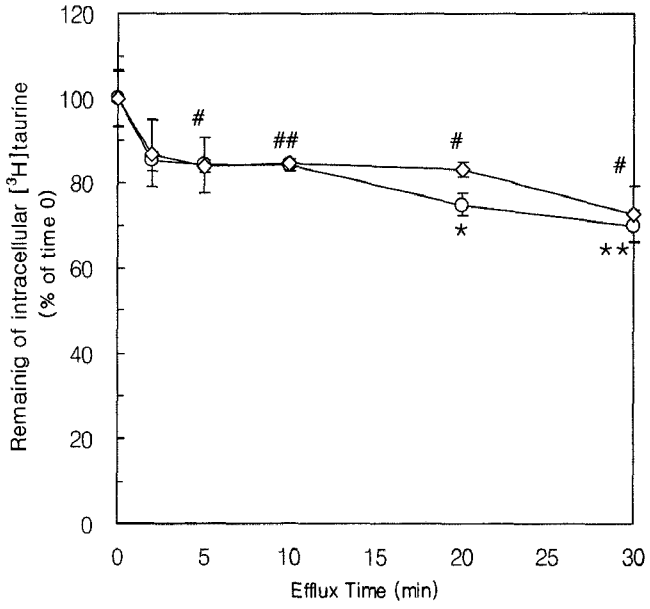

Figure 5–Time-course of [^{3}H]taurine efflux in TR-TBT 18d-1 (○) and TR-TBT 18d-2 (◇) cells. The cells were incubated for 60 min at 37°C with ECF buffer containing [^{3}H]taurine (28 nM). The cells were washed and then incubated for 2, 5, 10, 20, 30 min at 37°C with ECF buffer. [^{3}H]Taurine remaining in the cells was measured. Each point represents the mean ± SEM (n=3-4). *p<0.05, **p<0.01 (18d-1), #p<0.05, ##p<0.01 (18d-2) significantly different from time=0.

uptake. LPS and DEM treatment did not affect taurine efflux significantly, but TNF-α treatment inhibited the taurine efflux

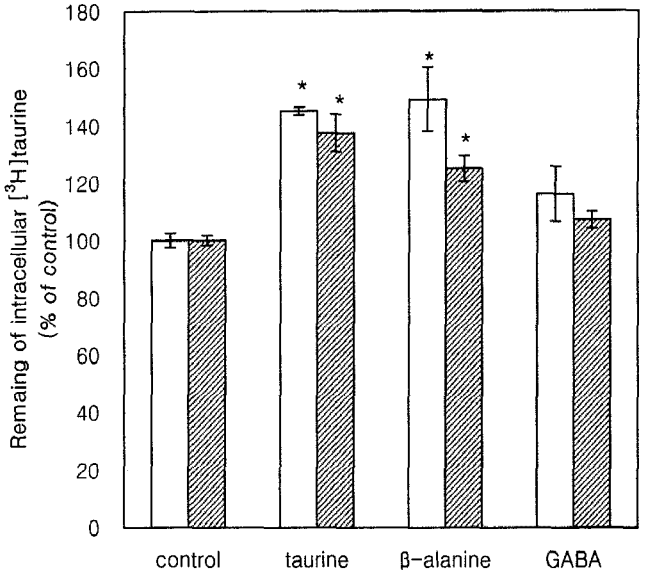

Figure 6–The inhibition of [^{3}H]taurine efflux by TauT substrates in TR-TBT 18d-1 (□), 18d-2 (▨) cells. The cells were incubated for 60 min at 37°C with ECF buffer containing [^{3}H]taurine (28 nM). The cells were washed and then incubated with ECF buffer added 1 mM β-alanine, 1 mM taurine or 1 mM GABA for 30 min. Remaining of [^{3}H]taurine in the cells was measured. Each value represents the mean ± SEM (n=3-4). *p<0.05 significantly different from normal efflux.

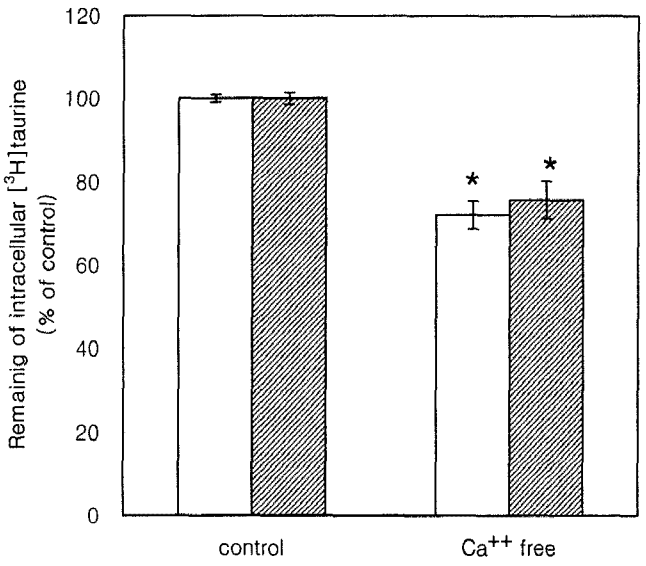

Figure 7–Effect of calcium ion on [^{3}H]taurine efflux by TR-TBT 18d-1 (□), 18d-2 (▨) cells. Under Ca^{++} free condition, Ca^{++} was replaced with Mg^{++}. Each value represents the mean ± SEM (n=3-4). *p<0.01 significantly different from normal efflux.

significantly (Fig. 8).

Discussion

In the present study, we investigated the change of taurine

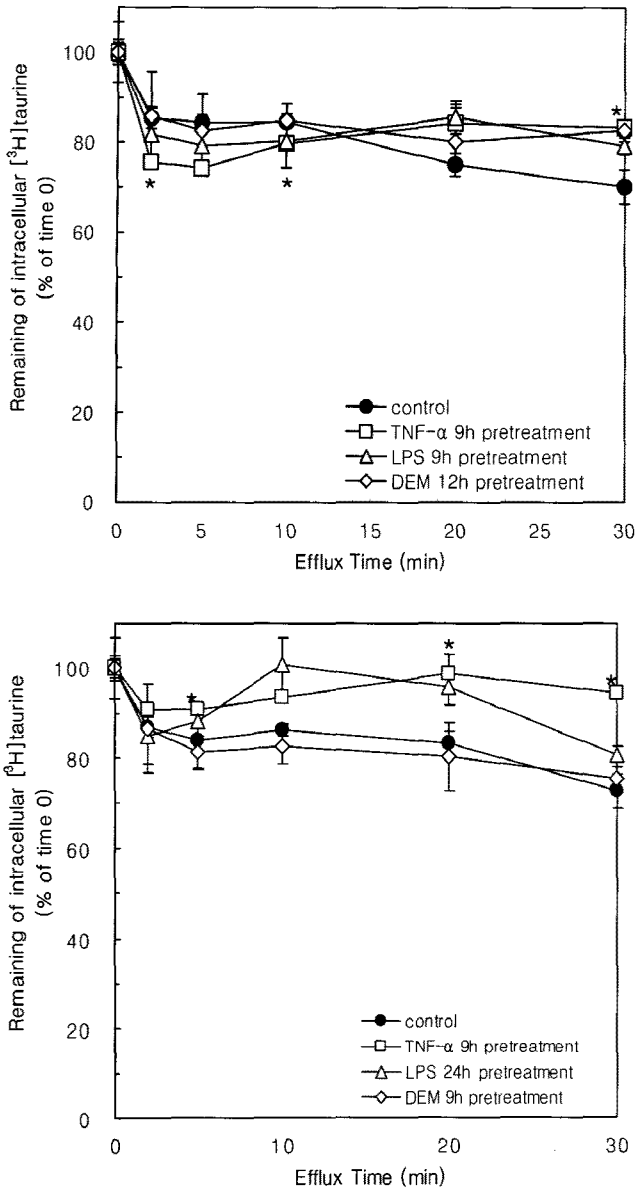

Figure 8—Time-course of [^{3}H]taurine efflux under oxidative stress by TR-TBT 18d-1 (upper), 18d-2 (lower) cells. Cells were treated with several stimulants for the specific time showed maximal taurine uptake, TNF-α 20 ng/mL for 9 h, LPS 10 ng/mL for 9 h and DEM 100 μM for 12 h (18d-1) and 9 h, 24 h and 9 h (18d-2) and then [^{3}H]taurine efflux was examined. Each value represents the mean ± SEM (n=3-4). *$p<0.05$ significantly different from normal efflux.

transport through the placenta under oxidative stress using conditionally immortalized rat syncytiotrophoblast cell, TR-TBT cells. It was reported that taurine transporter (TauT) existed in human placenta[8] and also the presence of TauT in TR-TBT cell was identified.[14] We also identified the characteristics of TauT which were previously known in various cells and organs such as brain, retina and intestinal cell[2] from the results (Fig. 1, Table I, II). The taurine uptake had time and sodium and chloride ion dependency and substrate specificity in TR-TBT cells. The taurine uptake had calcium ion dependency as well as sodium and chloride ion, and it was sensitive to calcium depletion in extracellur fluid than calcium abundance (Table III). The decrease of taurine uptake under calcium depletion was supported by the result of taurine efflux experiment under calcium free condition. The taurine efflux was evoked in calcium free condition about 20% over control (Fig. 7). An increase of taurine efflux might contribute to a decrease of taurine uptake at calcium free condition. In addition, Na^+-Ca^{++} exchanger is associated with these calcium effects on taurine transport.[4] A change of calcium ion concentration at intra or extracellular environment induced change of sodium ion concentration, and it may affect taurine transport because taurine transporter is very sensitive to sodium ion concentration. Also, it was reported that calcium free medium enhanced the taurine efflux in the rat striatum *in vivo*.[16] According to the study, the mechanism of an increase of taurine efflux by calcium depletion was considered that decreased calcium influx and increased nonspecific influx of sobium ion to the cells induced the taurine efflux evoked.[16]

Taurine acts as an antioxidant, and has protection function when cell is damaged by oxidative stress.[2] This study showed that taurine uptake was increased under oxidative stress conditions for 24 h, TNF-α, LPS and DEM, respectively pro-inflammatory cytokine, bacterial endotoxin and a compound inducing oxidative stress by depleting of glutathione levels (Fig. 2). It was reported that mRNA level of TauT was increased and taurine uptake was increased in blood brain-barrier[17] and intestinal cells[15] in treatment of TNF-α. The TNF-α signal activated nuclear factor-κB (NF-κB) transcriptional activity by nuclear translocation and the NF-κB binding site is found in the TauT promoter region.[18] Therefore, the TNF-α-NF-κB pathway could be associated in TauT up-regulation. DEM induced oxidative stress through the depletion of intracellular glutathione (GSH)[19] and up-regulated the cystine uptake in the organ.[20] Taurine is also sulfur-containing amino acid like cystine, therefore, it seems that DEM may induce an increase of taurine uptake in TR-TBT cells. It was reported that LPS reduced taurine uptake significantly in macrophage cell lines and Caco2 cells,[21-23] had no effect in conditionally immortalized brain microvascular endothelial cells.[17] In macrophage cell line, it was explained that LPS decreased taurine uptake might be via the action of nitric oxide.[21] In the present study, LPS increased taurine uptake slightly in TR-TBT cells, and it is contrary to previous results. Under hypertonic conditions, LPS instead activates taurine transport.[22] It is possible therefore to speculate that a switch controls the direction of LPS effect, but the complexity of the signaling pathways

makes it very difficult.[22] The mechanism study of changes of taurine transport by LPS is needed to be further elucidated.

On the other hand, treatment of hydrogen peroxide induced reduction of taurine uptake at a concentration of 2 mM and did not affect at a lower concentration of 100 μM (Fig. 4). Meng and colleagues showed that hydrogen peroxide elevated the phosphorylation of tyrosine residues via inhibition of a protein tyrosine phosphatase.[24] In general, taurine transport is regulated by protein kinase C (PKC) activity, because that TauT contains several serine and threonine residues for phosphorylation by PKC, so activation of PKC inhibits taurine transport.[2,3] We identified that taurine uptake was inhibited by PKC activator and phorbol 12-myristate 13-acetate (PMA) in TR-TBT cells (Table II). Accordingly, an increased phosphorylation of TauT could be involved in H_2O_2-induced reduction of taurine uptake in TR-TBT cells.

Exposing the cells to nitric oxide donor induced a reduction of taurine uptake significantly in TR-TBT cells (Fig. 4). NO donor, SIN-1, releases NO, O_2^- and $ONOO^-$. It was demonstrated that $ONOO^-$ interacted with TauT tyrosine residues and formation of nitrotyrosine was detected highly in NO donor treatment.[25-28] These alterations of transporter induced a reduction of taurine transport activity. In pregnant women having intrauterine growth restriction (IUGR) disease, placental transport capacity of taurine was reduced and fetal taurine level was decreased.[29] In addition, it has been previously shown that IUGR is associated with increased fetoplacental NO levels.[12] Thus, NO could play an important role in down-regulating TauT activity in IUGR.

Taurine efflux in TR-TBT cells was examined by measuring the remaining intracellular taurine. Taurine efflux from the cells existed as well as taurine uptake and TauT was involved in the efflux because taurine efflux was inhibited by TauT substrates (Fig. 6). While taurine uptake was increased in TNF-α, LPS and DEM pretreatment, one of the oxidative stress stimulations, TNF-α, affected taurine efflux significantly. Taurine uptake was increased to the maximum level and taurine efflux was inhibited in exposure to TNF-α for 9 h treatment. These results of the TNF-α affecting on taurine efflux coincided with previous *in vivo* study in brain cell.[30] Thus, oxidative stress condition affected taurine efflux from TR-TBT cells as well as taurine uptake.

Acknowledgements

The authors wish to thank Dr. E. Nakashima for providing TR-TBT cell lines with kindness.

References

1) Huxtable, R. J., Physiological actions of taurine, *Physiol. Rev.*, **72**, 101-163 (1992).
2) Tappaz, M. L., Taurine biosynthetic enzymes and taurine transporter: molecular identification and regulations, *Neurochem. Res.*, **29**, 83-96 (2004).
3) Lambert, I. H., Regulation of the cellular content of the organic osmolyte taurine in mammalian cells, *Neurochem. Res.*, **29**, 27-63 (2004).
4) Foos, T. M. and Wu, J. Y., The role of taurine in the central nervous system and the modulation of intracellular calcium homeostasis, *Neurochem. Res.*, **27**, 21-26 (2002).
5) Chapman, G. E. and Greenwood, C. E., Taurine in nutrition and brain development, *Nutr. Res.*, **8**, 955-968 (1988).
6) Sturman, J. A., Taurine in development, *J. Nutr.*, **118**, 1169-1176 (1988).
7) Jansson, T., Amino acid transporters in the human placenta, *Pediatr. Res.*, **49**, 141-7 (2001).
8) Kulanthaivel, P., Cool, D. R., Ramamoorthy, S., Mahesh, V. B., Leibach, F. H. and Ganapathy, V., Transport of taurine and its regulation by protein kinase C in the JAR human placental choriocarcinoma cell line, *Biochem. J.*, 277, 53-58 (1991).
9) Ramamoorthy, S., Leibach, F. H., Mahesh, V. B., Han, H., Yang-Feng, T., Blakely, R. D. and Ganapathy, V., Functional characterization and chromosomal localization of a cloned taurine transporter from human placenta, *Biochem. J.*, **300**, 893-900 (1994).
10) Rubin, E., Essential Pathology. Lippincott Williams & Wilkins, Maryland, (2001).
11) Norberg, S., Powell, T. L. and Jansson, T., Intrauterine growth restriction is associated with a reduced activity of placental taurine transporters, *Pdeiatr. Res.*, **44**, 233-238 (1998).
12) Lyall, F., Greer, I., Young, A. and Myatt, L., Nitric oxide concentrations are increased in the feto-placental circulation in intrauterine growth restriction, *Placenta*, **17**, 165-168 (1996).
13) Kitano, T., Iizasa, H., Terasaki, T., Asashima, T., Matsunaga, N., Utoguchi, N., Watanabe, Y., Obinata, M., Ueda, M., and Nakashima, E., Polarized glucose transporters and mRNA expression properties in newly developed rat syncytiotrophoblast cell lines, TR-TBTs, *J. Cell. Physiol.*, **193**, 208-218 (2002).
14) Kitano, T., Iizasa, H., Hwang, I. W., Hirose, Y., Morita, T., Maeda, T. and Nakashima, E., Conditionally immortalized syncytiotrophoblast cell lines as new tools for study of the blood-placenta barrier, *Biol. Pharm. Bull.*, **27**, 753-759 (2004).
15) Mochizuki, T., Satsu, H. and Shimizu, M., Signaling pathways involved in tumor necrosis factor α-induced upregulation of the taurine transporter in Caco-2 cells, *FEBS Letters*, **579**, 3069-3074 (2005).
16) Molchanova, S. M., Oja, S. S. and Saransaari P., Mechanisms of enhanced taurine release under Ca^{2+} depletion, *Neurochem. Int.*, **47**, 343-349 (2005).
17) Kang, Y. S., Ohtsuki, S., Takanaga, H., Tomi, M., Hosoya, K.

I. and Terasaki, T., Regulation of taurine transport at the blood-brain barrier by tumor necrosis factor-α, taurine and hypertonicity, *J. Neurochem.*, **83**, 1188-1195 (2002).
18) Han, X., Budreau, A. M. and Chesney, R. W., Cloning and characterization of the promoter region of the rat taurine transporter (TauT) gene, *Adv. Exp. Med.*, **483**, 97-108 (2000).
19) Bannai, S., Induction of cystine and glutamate transport activity in human fibroblast by diethyl malteate and other electrophilic agents, *J. Biol. Chem.*, **289**, 2435-2440 (1984).
20) Hosoya, K., Saeki, S., and Terasaki, T., Activation of carrier-mediated transport of L-cystine at the blood-brain and blood-retinal barriers in vivo, *Microvasc. Res.*, **62**, 136-142 (2001).
21) Kim, H.W., Kim, J.H., An, H.S., Park, K.K., Kim, B.K. and Park, T., Myo-inositol restores the inflammation-induced down-regulation of taurine transport by the murine macrophage cell line, RAW 264.7, *Life Sci.*, **73**, 2477-2489 (2003).
22) Romio, L., Zegarra-Moran, Varesio, L and Galietta, L.J.V., Regulation of taurine transport in murine macrophages, *Amino Acids*, **21**, 151-160 (2001).
23) O'Flaherty, L., Stapleton, P.P., Redmond, H.P. and Bouchier-Hayes, D., Dexamethasone and lipopolysaccharide regulation of taurine transport in Caco-2 cells, *J. Surg. Res.*, **69**, 331-336 (1997).
24) Meng, T. C., Fukada, T. and Tonks, N. K., Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo, *Mol. Cell*, **9**, 387-399 (2002).
25) Kulanthaivel, P., Leibach, F. H., Mahesh, V. B. and Ganapathy, V., Tyrosine residues are essential for the activity of the human placental taurine transporter, *Biochem. Biophys. Acta.*, **985**, 139-146 (1989).
26) Myatt, L., Rosenfield, R. B., Eis, A. L., Brockman, D. E., Greer, I. and Lyall, F., Nitrotyrosine residues in placenta. Evidence of peroxynitrite formation and action, *Hypertension*, **28**, 488-493 (1996).
27) Roggensack, A. M., Zhang, Y. and Davidge, S. T., Evidence for peroxynitrite formation in the vasculature of women with preclampsia, *Hypertension*, **33**, 83-89 (1999).
28) Khullar, S., Greenwood, S. L., Mccord, N., Glazier, J. D. and Ayuk, P. T. Y., Nitric oxide and superoxide impair human placental amino acid uptake and increase Na^+ permeability: implications for fetal growth, *Free radical Biolocy & Medicine.*, **36**, 271-277 (2004).
29) Roos, S., Powell, L. and Jansson, T., Human placental taurine transporter in uncomplicated and IUGR pregnancies: cellular localization , protein expression, and regulation, *Am. J. Physiol. Regul. Inter. Comp. Physiol.*, **287**, R886-R893 (2004).
30) Lee, N.Y. and Kang, Y. S., The brain-to-blood efflux transport of taurine and changes in the blood-brain barrier transport system by tumor necrosis factor-α, *Brain Res.*, **1023**, 141-147 (2004).