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Ionomycin Recovers Taurine Transporter Activity in Cyclosporin A Treated Macrophages

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Abstract

Taurine is a major β-amino acid in various tissues. Taurine transporter (TAUT) is responsible for the transportation of taurine in the cell. The transporter is affected by various stimuli to maintain its cell volume. Macrophage cell volume varies in its activated states. In our experiment, it was found that the murine macrophage cell line, RAW264.7, expressed TAUT protein in its membrane. Its transportation activities could be blocked by a β-amino acid such as β-alanine, but not by α-amino acids in this cell line. When assessed in RAW264.7 under the influence of immunosuppressive reagents, the activity of the TAUT was decreased by the treatment of rapamycin (RM) or cyclosporin A (CsA). However when ionomycin (IM) was added to this system, TAUT activity was recovered only in CsA-treated cells in a concentration-dependent manner. In order to inhibit the voltage gated Ca^{+2} channel, calmidazolium was added to the RAW264.7 cell line. Treatment of the cell with calmidazolium completely blocked TAUT. Furthermore, addition of IM to this system recovered the activity of TAUT again. When we added phorbol myristate acetate (PMA) to the cell line, secretion of nitric oxide (NO) was increased 4-fold and the TAUT activity was decreased 5-fold. However, the addition of N-nitro L-arginine methyl ester (L-NAME), an inducible NO synthase (iNOS) inhibitor, to the PMA-treated cells, resulted in the recovery of TAUT activity. These results showed that the activity of TAUT was sensitive to the intracellular concentrations of both Ca^{+2} and NO.

Key words: taurine transporter, macrophage, cyclosporin A, rapamycin, nitric oxide

INTRODUCTION

Taurine (2-aminoethanesulfonic acid, $^{+}NH_3CH_2CH_2SO_3^{-}$) is one of the β-amino acids, which is biosynthesized from cysteine in mammarian animals. It is an abundant intracellular free amino acid in mammalian tissues, and an energy-dependent, cell membrane-associated transport mechanism is responsible for the maintenance of a high intracellular taurine concentration in most tissues. Such high concentrations of taurine in various tissues suggest that it exerts important functions such as neuromodulation (1), antioxidation (2,3), and osmoregulation (4). The taurine transporter (TAUT) was cloned from human (5), mouse (6), rat (7), dog (8), and pig (9) DNA. Murine TAUT was cloned from mouse brain cDNA library. The deduced amino acid sequence was 590 amino acids with typical characteristics of sodium-dependent neurotransmitter transporters. TAUT is abundant in proximal tubules in which β-amino acids such as β-alanine, β-aminobutyric acid and gamma-aminobutyric acid (GABA) are actively reabsorbed through this transporter (10-13). TAUT was found to be a Na^{+}/Cl^{-} cotransport system where the ratio of transportation of taurine : Na^{+} : Cl^{-} was 1 : 2 : 1 (13,14).

Protein kinase C plays central roles in signal transduction and the regulation of transportation/absorption of nutrients. Inhibition of TAUT by the stimulation of PKC was first reported by Kulanthaivel et al. (15) in the placental choriocarcinoma cell line. Inhibition of TAUT due to the phosphorylation by PKC has been reported in the kidney (16), colon carcinoma (17) and murine macrophage (18) cell lines. Since the discovery of ionomycin as a calcium ionophore (19), it increases the intracellular Ca^{2+} concentration that leads to the activation of the cell. The blocking of TAUT could be affected by calmoduline (20). Inhibition of TAUT by cyclosporin A, an immunosuppressive agent, could be recovered by W-7, a calmoduline antagonist or calmidazolium. However another immunosuppressive agent, FK506, does not affect TAUT activity (21). Blocking of TAUT by PMA could be recovered by addition of staurosporine, a PKC inhibitor. However, this recovery was not affected by protein synthesis inhibitors such as cyclohexamide, actinomycin D, colchicine and cytochalasin D (17), suggesting that the TAUT was not induced, but expressed constitutively on the cell.

In this study we investigated the effect of immunosuppressive agents such as cyclosporin A and rapamycin on the TAUT activity, and found these agents blocked the TAUT activity of the murine macrophage. And we also found that ionomycin could recover the blockade of TAUT activity in cyclosporin A treated cells, but not in rapamycin treated cells.

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MATERIALS AND METHODS

Cell line and chemicals

The Macrophage RAW264.7 cell line which is an Abelson leukemia virus-transformed murine macrophage cell line, was obtained from the American Type Culture Collection (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM) and [2-^{3}H(N)]-Taurine (21.9 Ci/mmol) were bought from Gibco-BRL (Grand Island, NY) and NEN (Boston, MA), respectively. Fetal bovine serum (FBS), penicillin and streptomycin, dexamethasone (DM), PMA, taurine, β-alanine, L-serine, L-leucine, rapamycin, EDTA, calmidazolium, staurosporine, ionomycin (IM) and N-nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma Chemical (St. Louis, MO). Interferon-γ was obtained from R&D Systems (Minneapolis, MN, USA). Cyclosporin A (CsA, Sandoz) was kindly provided by Han-mi Pharmaceutical Co., Korea.

Cell culture and taurine transportation

RAW264.7 cells were cultured and maintained in 90 mm culture dishes in a DMEM medium containing 10% heat-inactivated fetal bovine serum (FBS) and penicillin (100 U/ml) /streptomycin (100 μg/ml). Cultures were maintained at 37°C in a 5% CO_2 incubator until they reached confluence. The taurine transportation study was performed according to the previous report (22), *i.e.*, when the cells were confluent in 6-well plates, the medium was removed from the monolayer culture and replaced with a 2 ml transporter buffer, which contained 25 mM-HEPES/Tris (pH 7.5), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM D-glucose. After stabilizing the cells for 30 min at 37°C, 1 μCi of [^{3}H]-taurine was added to each culture and incubated for 30 min. Then the cells were washed thrice with ice-cold Na^+ deficient buffer and lyzed with 1 ml of 0.2 N NaOH/0.5% SDS. The lysate was mixed with 2 ml scintillation cocktail solution and its radioactivity was measured by a liquid scintillation counter (Hewlett-Packard, 2500TR).

Cell stimulation

For the stimulation of the RAW264.7 cells, confluent monolayer cells in DMEM containing 10% fetal bovine serum were treated with rafamycin, ionomycin, CsA, calmidazolium, PMA or L-NAME for 30 min in 6-well plates by using a transportation buffer. Then the cells were treated with 1 μCi of [^{3}H]-taurine for another 30 min and washed thrice with a cold buffer followed by lyzing with the lysis buffer. The transported radioactive taurine in the lysate was measured by a β-counter. For the analysis of nitric oxide, RAW cells were stimulated with PMA, CsA or ionomycin for 48 hrs. The resulting supernatants were obtained by centrifugation at 2,000 rpm. One hundred μl of the supernatant were transferred to the ELISA titer plate and mixed with 100 μl of Griess reagent that was mixed 1:1 (v/v) with 1% sulfanylamide in 2% phosphoric acid and 0.2% N-naphthylethylenediamine 2HCl in distilled water for 10 min at room temperature (23). An ELISA reader at 540 nm was used for the measurement of absorbance, together with a standard sodium nitrite solution.

RESULTS AND DISCUSSION

Substrate specificity of taurine transporter on RAW cell

The murine macrophage cell line, RAW264.7, was used for the measurement of TAUT activity. Confluent cells on 6-well plate was pretreated with unlabelled α- or β-amino acids at a concentration of 1 mM for 10 min and followed by the addition of 1 μCi [^{3}H]-taurine for another 30 min. Pretreatment of the cell with cold taurine for 30 min led to the blocking of hot taurine transportation to 4.8% of the control value. Pretreatment with β-alanine also blocked hot taurine transportation to 12.9% of the control value. However, pretreatment of the cell with α-amino acids such as L-serine or L-leucine did not induce hot taurine transportation (Table 1). From these experiments murine macrophages were confirmed to express β-amino acid specific transporter on the cell membrane. As pretreatment with non-radioactive taurine prevented radioactive taurine transportation, this transporter seems to be TAUT. Furthermore TAUT was confirmed by the properties of the dependence on the presence of the Na^+ ion for the uptake of taurine by the RAW cells.

Inhibition of taurine transporter activity by rapamycin

RAW264.7 cells were exposed to immunosuppressive agents such as rapamycin and CsA for 5 min, and then measured for the activity of the TAUT. One ng/ml of rapamycin was the minimal inhibitory concentration of the TAUT activity when we used 1, 10, 100 and 1,000 ng/ml rapamycin on this system. At the concentration of 1 ng/ml of rapamycin, TAUT activity was reduced to 11.9% when compared to that of the control (Fig. 1). The blocking of the transporter activity could be induced within 5 min of the incubation with rapamycin. When the same experiment was done with CsA, minimal concentration and time for the blocking effect of the TAUT were 5 nM and 5 min, respectively (data not shown). The blocking pattern of CsA was almost similar to that of the rapamycin, suggesting that a high intake of taurine is required to maintain a normal concentration of taurine in the cell during the period of taking immunosuppressive agents. This blocking phenomenon associated with the inhibition of

Table 1. Substrate specificity of the carrier system on taurine uptake

Pretreated reagent	[^{3}H]-Taurine transportation in cpm and %	
PBS	391,412±5,810[1)]	100.0%
1 mM Taurine	18,943±83	4.8%
1 mM β-Alanine	50,543±1,697	12.9%
1 mM L-Serine	400,575±2,367	102.3%
1 mM L-Leucine	438,381±1,125	111.9%

RAW264.7 cell line was stabilized with transporter buffer for 30 min in a 6-well plate and then pretreated with the reagent for 10 min. Then added 1 μCi [^{3}H]-taurine for 30 min. After lysis, intracellular [^{3}H]-taurine was measured by a liquid scintilation counter

[1)]Values are mean±SD from two separate experiments.

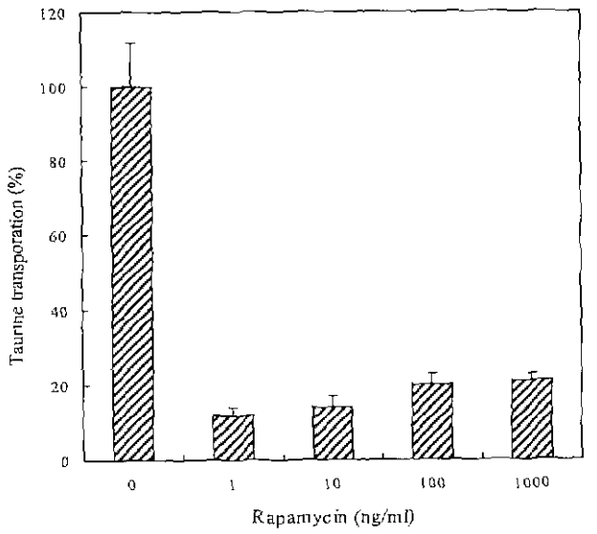

Fig. 1. Effects of rapamycin on taurine transpoter activity in RAW264.7 cell line.

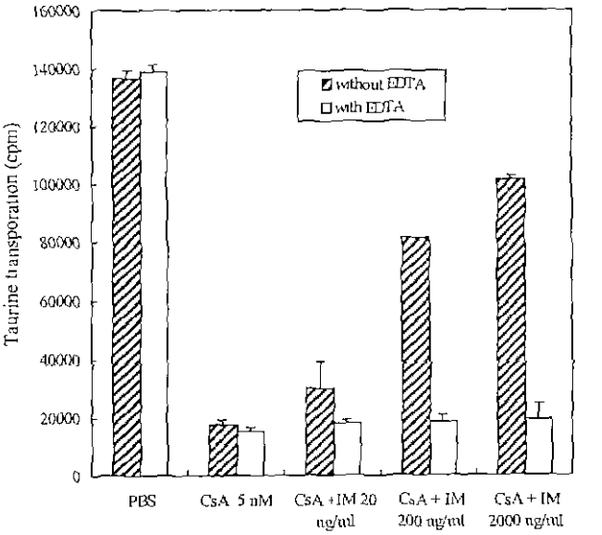

Fig. 2. Pretreatment effects of cytosolic Ca^{2+} chelator, EDTA on taurine transporter activity in CsA and IM treated cells. CsA, cyclosporin A; IM, ionomycin.

ATP-dependent taurocholate transportation by CsA, which results in the cholestasis as a side effect of CsA therapy (24). Immunosuppressants FK506 (tacrolimus) and CsA in rat hepatocyte (25) and HeLa cell (26) were reported to inhibit function of the multidrug efflux pump that causes certain multidrug resistance to anticancer drugs (27).

Recovery of taurine transporter activity by ionomycin

Since intracellular concentration of Ca^{+2} is important for the activation of macrophages, RAW264.7 cells were co-treated with rapamycin and ionomycin (IM) simultaneously. The concentration of rapamycin was fixed at 1 ng/ml, and the concentration of IM was increased 100-fold from 20 to 2,000 ng/ml. When the concentration of IM was increased in rapamycin-treated cells, the recovery of the TAUT activity was not altered by the addition of IM (Table 2). However in the case of co-treatment with CsA and IM, increasing IM with concentration recovered TAUT activity in CsA-treated cells (Fig. 2). Treatment of the RAW cells with 5 nM CsA reduced the taurine transport activity (17,268±1,763 cpm) to 12.6% of the value for the control (136,598±3,112 cpm). When IM was added to this system at concentrations of 20, 200 and 2,000 ng/ml, the TAUT activity was increased to 21.7% (29750 ±9288 cpm), 59.4% (81,235±134 cpm) and 74.1% (101,252 ±1,357 cpm), respectively. These results indicate that an increase in intracellular Ca^{+2} concentration activates TAUT activity.

Table 2. Effect of ionomycin on taurine transporter activity in the rapamycin-treated RAW264.7 cells

Stimulator	[^{3}H]-Taurine transportation in cpm and %	
PBS	200,431±1,323[1)]	100.0%
RM (1 ng/ml)	21,465±4,764	10.7%
RM (1 ng/ml)+IM (20 ng/ml)	21,281± 588	10.6%
RM (1 ng/ml)+IM (200 ng/ml)	23,469±2,198	11.7%
RM (1 ng/ml)+IM (2,000 ng/ml)	25,356±1,741	12.6%

RAW264.7 cells were stabilized with the transportation buffer for 30 min and stimulated for 10 min. Then added 1 μCi [^{3}H]-taurine for 30 min. RM, rafamycin; IM, ionomycin.

[1)]Values are mean±SD from three separate experiments.

To prove the involvement of intracellular Ca^{+2} on the recovery of TAUT activity, EDTA, Ca^{+2} chelator, was added to the above system. When 800 nM EDTA was added to the cell systems that were treated with 5 nM CsA+20 ng/ml IM, 5 nM CsA+200 ng/ml IM, and 5 nM CsA+2,000 ng/ml IM, the corresponding TAUT activities were 12.8% (17,786± 1,244 cpm), 13.2% (18,333±2,447 cpm) and 13.9% (19,294 ±5,224 cpm) of the control value (138,600±2,510 cpm), respectively (Fig. 2). This result indicates that TAUT activity is affected by the presence of intracellular Ca^{+2}. Therefore intracellular Ca^{+2} appears to be essential for the recovery of the TAUT activity that was blocked by CsA. Our conclusion seems to be in conflict with the observations that extracellular Ca^{-2} inactivated TAUT (28). The discrepancy may be due to two reasons. First, in our experiment we measured the recovery activity. So it may be different from the activity of the transporter. The second reason may be due to the difference of the cell lines used. The TAUT of murine macrophages may be different from that of the human placental brush border membrane.

To prove the involvement of calmodulin on the modulation of the TAUT activity, calmidazolium, inhibitor of calmodulin, was added to the RAW cell. When 5 μg/ml of calmidazolium was added to the cell, taurine transportation activity was 3.9% of that of the control value, indicating that calmodulin is responsible for the activity of TAUT. However when 20 ng/ml of IM was added to this system, TAUT activity was recovered to 64% of that of the control value, suggesting the involvement of intracellular Ca^{+2} for the recovery of taurine transporter activity (Table 3). The effect of the calmodulin

Table 3. Effect of the calmodulin antagonist on taurine transporter activity in RAW264.7 cells

Stimulator	[^{3}H]-*Taurine transportation in cpm and %*	
PBS	300,693±3,124[1)]	100.0%
CsA	11,379± 978	3.7%
Calmidazolium	11,786± 513	3.9%
CsA+Calmidazolium	11,542±1,003	3.8%
Calmidazolium+IM	192,543±2,668	64.0%

RAW264.7 cells were stabilized with transportation buffer for 30 min and stimulated for 12 hrs. Then added 1 μCi [^{3}H]-taurine for 30 min. Final concentrations of CsA, calmidazolium and IM were 5 nM, 5 μg/ml and 20 ng/ml, respectively. CsA, cyclosporin A; IM, ionomycin.
[1)]Values are mean±SD from three separate experiments.

inhibitor on the TAUT activity is coincident with the result of Ramamoorthy et al. (29). They demonstrated that decrease of the TAUT activity by the treatment of retinal pigment cell line with calmodulin antagonists such as CGS 9343 B (CGS) or W-7 was accompanied by a decrease in the maximal velocity of the transporter.

Nitric oxide blocks taurine transporter activity

Activation of protein kinase C (PKC) was known to inhibit TAUT activity. Addition of 10 ng/ml of PMA to the RAW cell reduced TAUT activity to 19.4% of that of the control. When L-NAME, an inhibitor of NOS, was added to this culture system, TAUT activity was recovered to 60.7% of that of the control value, indicating that blocking of the production of NO could induce recovery of TAUT activity (Tables 4 and 5). During the inflammation NO production is increased in the cytosol of the macrophage. The macrophage may require large quantities of taurine to neutralize the excess NO in the cytosol to prevent damage by the NO. Inhibition of NO production was also dependent on intracellular taurine concentration (30).

Table 4. Effect of the iNOS inhibitor, L-NAME. on taurine transporter activity in RAW264.7 cell line

Stimulator	[^{3}H]-Taurine transportation in cpm and %	
PBS	165,949±2,835[1)]	100.0%
PMA	32,210±1,498	19.4%
L-NAME	204,738±1,389	123.0%
PMA+*DM*	106,369±2,081	64.0%
PMA+L-NAME	100,694±1,085	60.7%

PMA, phorbol myristate acetate; L-NAME, N-nitro L-arginine methyl ester; *DM, dexamethasone.*
[1)]Values are mean±SD from three separate experiments.

Table 5. Effect of PMA and ionomycin on the nitric oxide production in RAW264.7 cell line

Stimulator	Nitrite concentration (μM)	Percent (%)
PBS	6.2±1.8[1)]	100
PMA	25.8±2.8	416
IM	5.0±3.1	80
CsA	17.5±4.0	282
PMA+IM	9.4±4.0	151
CsA+IM	12.5±0.9	201

PMA, phorbol myristate acetate; IM, ionomycin: CsA, cyclosporin A
[1)]Values are mean±SD from three separate experiments.

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REFERENCES

1. Oja, S.S. and Kontro, P. . Neuromodulatory and trophic actions of taurine. *Prog. Clin. Biol. Res.*, **351**, 69 (1990)
2. *Sawamura*, A., Azuma, J., Awata, N., Harada, H. and Kishimoto, S. : Modulation of cardiac Ca^{++} current by taurine. *Prog. Clin. Biol. Res.*, **351**, 207 (1990)
3. Gordon, R. E., Heller, R. F. and Heller, R. F. · Taurine protection of lungs in hamster models of oxidant injury: a morphologic time study of paraquat and bleomycin treatment. *Adv. Exp. Med. Biol.*, **315**, 319 (1992)
4. Koyama, I., *Nakamura*, T., *Ogasawara*, M., Nemoto, M. and Yoshida, T : The protective effect of taurine on the biomembrane against damage produced by the oxygen radical. *Adv. Exp. Med. Biol.*, **315**, 355 (1992)
5. Jhiang, S. M., Fithian, L., Smanik, P., McGill, J., Tong, Q. and Mazzaferri, E. L. : Cloning of the human taurine transporter and characterization of taurine uptake in thyroid cells. *FEBS Lett.*, **318**, 139 *(1993)*
6. Liu, Q. R., Lopez-Corcuera, B., Nelson, H., Mandiyan, S. and Nelson, N. : Cloning and expression of a cDNA encoding the transporter of taurine and beta-alanine in mouse brain. *Proc. Natl. Acad. Sci. USA*, **89**, 12145 (1992)
7. Smith, K. E., Borden, L A., Wang, C. H., Hartig, P. R., Branchek, T. A. and Weinshank, R. L. : Cloning and expression of a high affinity taurine transporter from rat brain. *Mol. Pharmacol.*, **42**, 563 (1992)
8. Uchida. S., Kwon, H. M., Yamauchi, A., Preston, A. S., Marumo, F. and Handler, J. S : Molecular cloning of the cDNA for an MDCK cell Na (+)- and Cl (-)-dependent taurine transporter that is regulated by hypertonicity. *Proc. Natl. Acad. Sci. USA*, **89**, 8230 (1992)
9. Han, X., Budreau, A. M. and Chesney, R. W. : Molecular cloning and functional expression of an LLC-PK1 cell taurine transporter that is adaptively regulated by taurine. *Adv. Exp. Med. Biol.*, **442**, 261 (1998)
10. Chesney, R. W., Gusowski, N., Dabbagh, S., Theissen, M., Padilla, M. and Diehl, A. : Factors affecting the transport of beta-amino acids in rat renal brush-border membrane vesicles. The role of external chloride. *Biochim. Biophys. Acta*, **812**, 702 (1985)
11. Chesney, R. W., Gusowski, N., Zeilkovic, I. and Padilla, M. : Developmental aspects of renal beta-amino acid transport. V: Brush border membrane transport in nursing animals-effect of age and diet. *Pediatr. Res.*, **20**, 890 (1986)
12. Chesney, R. W., Gusowski, N. and Zelikovic, I. : Developmental aspects of renal beta-amino acid transport. VI. The role of membrane fluidity and phospholipid composition in the renal adaptive response in nursing animals. *Pediatr. Res.*, **22**, 163 (1987)
13. Chesney, R. W., Zelikovic, I., Friedman, A. L., Dabbagh, S., Lippincott, S., Gusowski, N. and Stjeskal-Lorenz, E. : Renal taurine transport-recent developments. *Adv. Exp. Med Biol.*,

217, 49 (1987)
14. Zelikovic, I., Stejskal-Lorenz, E., Lohstroh, P., Budreau, A. and Chesney, R. W. : Anion dependence of taurine transport by rat renal brush-border membrane vesicles. *Am. J. Physiol.*, **256** (4 Pt 2), F646 (1989)
15. 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** (Pt 1), 53 (1991)
16. Jones, D. P., Miller, L. A., Dowling, C. and Chesney, R. W. : Regulation of taurine transporter activity in LLC-PK1 cells: role of protein synthesis and protein kinase C activation. *J. Am. Soc. Nephrol.*, **2**, 1021 (1991)
17. Brandsch, M., Miyamoto, Y., Ganapathy, V. and Leibach, F. H. : Regulation of taurine transport in human colon carcinoma cell lines (HT-29 and Caco-2) by protein kinase C. *Am J. Physiol.*, **264** (5 Pt 1), G939 (1993)
18. Kim, H. W., Shim, M. J., Kim, W. B. and Kim, B. K. : Regulation of taurine transporter activity by glucocorticoid hormone. *J. Biochem. Mol. Biol.*, **28**, 527 (1995)
19. Liu, C. and Hermann, T. E. : Characterization of ionomycin as a calcium ionophore. *J. Biol. Chem.*, **253**, 5892 (1978)
20. Ramamoorthy, S., Del Monte, M. A., Leibach, F. H. and Ganapathy, V. : Molecular identity and calmodulin-mediated regulation of the taurine transporter in a human retinal pigment epithelial cell line. *Curr. Eye Res.*, **13**, 523 (1994)
21. Ramamoorthy, S., Leibach. F. H., Mahesh, V. B. and Ganapathy, V. : Selective impairment of taurine transport by cyclosporin A in a human placental cell line. *Pediatr. Res.*, **32**, 125 (1992)
22 Kim, H. W., Lee, E. J., Shim, M. J. and Kim, B. K. . Effects of steroid hormones and cyclosporin A on taurine-transporter activity in the RAW264.7 cell line. *Adv. Exp. Med. Biol.*, **442**, 247 (1998)
23. Park, E., Quinn, M. R., Wright, C. E. and Schuller-Levis, G. : Taurine chloramine inhibits the synthesis of nitric oxide and the release of tumor necrosis factor in activated RAW 264.7 cells. *J. Leukoc. Biol.*, **54**, 119 (1993)
24. Bohme, M., Jedlitschky, G., Leier, I., Buchler, M. and Keppler, D. : ATP-dependent export pumps and their inhibition by cyclosporins. *Adv. Enzyme Regul.*, **34**, 371 (1994)
25. Takeguchi, N., Ichimura, K., Koike, M., Matsui, W., Kashiwagura, T. and Kawahara, K. : Inhibition of the multidrug efflux pump in isolated hepatocyte couplets by immunosuppressants FK506 and cyclosporin. *Transplantation*, **55**, 646 (1993)
26. Kirk, J. and Kirk, K. : Inhibition of volume-activated I- and taurine efflux from HeLa cells by P-glycoprotein blockers correlates with calmodulin inhibition. *J. Biol. Chem.*, **269**, 29389 (1994)
27. Wigler, P. W. and Patterson, F. K. : Inhibition of the multidrug resistance efflux pump *Biochim. Biophys. Acta*, **1154**, 173 (1993)
28. Kulanthaivel, P., Miyamoto, Y., Mahesh, V. B., Leibach, F. H. and Ganapathy, V. : Inactivation of taurine transporter by calcium in purified human placental brush border membrane vesicles. *Placenta*, **12**, 327 (1991)
29. Ramamoorthy, S., Del Monte, M. A., Leibach, F. H. and Ganapathy, V. : Molecular identity and calmodulin-mediated regulation of the taurine transporter in a human retinal pigment epithelial cell line. *Curr Eye Res*, **13**, 523 (1994)
30. Park, E., Schuller-Levis, G., Jia, J. H. and Quinn, M. R. : Preactivation exposure of RAW 264.7 cells to taurine chloramine attenuates subsequent production of nitric oxide and expression of iNOS mRNA. *J. Leukoc. Biol.*, **61**, 161 (1997)

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