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Regulation of Taurine Transporter Activity by Glucocorticoid Hormone

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Abstract: Human taurine transporter has 12 transmembrane domains and its molecular weight is 69.6 kDa. The long cytoplasmic carboxy and amino termini might function as regulatory attachment sites for other proteins. Six potential protein kinase C phosphorylation sites have been reported in human taurine transporter. In this report, we studied the effects of phorbol 12-myristate 13-acetate (PMA) and glucocorticoid hormone on taurine transportation in the RAW 264.7, mouse macrophage cell line. When the cells were incubated with [^{3}H]taurine in the presence or absence of Na^+ ion for 40 min at 37°C, the [^{3}H]taurine uptake rate was 780-times higher in the Na^+-containing buffer than in the Na^+-deficient buffer, indicating that this cell line expresses taurine transporter protein on the cell surface. THP1, a human promonocyte cell line, also showed a similar property. The [^{3}H]taurine uptake rate was not influenced by the inflammatory inducing cytokines such as interleukin-1, gamma-interferon or interleukin-1+gamma-interferon, but was decreased by the PMA in the RAW 264.7 cell line. This suggests that activation of protein kinase C inhibits taurine transporter activity directly or indirectly. The inhibition of [^{3}H]taurine uptake by PMA was time-dependent. Maximal inhibition occurred in one hr stimulation with PMA. Increasing the treatment time beyond one h reduced the [^{3}H]taurine uptake inhibition due to the depletion or inactivation of protein kinase C. The cell line also showed concentration-dependent [^{3}H]taurine uptake under PMA stimulation. The phorbol-ester caused 23% inhibition at the concentration of 1 μM PMA. The inhibition was significant even at a concentration as low as 10 nM PMA. The reduced [^{3}H]taurine uptake could be recovered by treatment with glucocorticosteroid hormone. Dexamethasone led to recover of the reduced taurine uptake induced by phorbol-ester, recovering maximally after one hr. This may suggest that macrophage cells require higher taurine concentration in a stressed state, for the secretion of glucocorticoid hormone is increased by hypothalamo-pituitary-adrenocortical (HPA) axis activation in the blood stream.
Key words: dexamethasone, macrophage, PKC, taurine transporter.

Taurine is one of the major intracellular amino acids in mammals. It is involved in a number of important physiological processes such as membrane stabilization, detoxification, antioxidation, osmoregulation, modulation of calcium flux and neuroregulation.

Recently there has been increasing interest in the mechanisms of taurine transportation in various tissues. Ehrlich cells increase taurine influx in an extracellular pH-dependent manner (Lambert and Hoffman, 1990). Rat brain synaptic membrane vesicles required Na^+ and Cl^- ions for taurine uptake (Iwata *et al.*, 1990). Taurine could be transported above the concentration of plasma osmolarity in toads (Boxter *et al.*, 1990). Therefore these properties support the presence of a taurine transporter in various tissues.

Cloning of cDNA encoding a transporter of taurine has been achieved in mouse brain cells (Liu *et al.*, 1992), rat brain cells (Smith *et al.*, 1992), canine kidney cells (Uchida *et al.*, 1992) and human thyroid cells (Jhiang *et al.*, 1993). The taurine transporter gene was found to be located in chromosome 3 p24~p26 in human placenta (Ramamoorthy *et al.*, 1994). This gene has a molecular weight of 69.6 kDa. The long cytoplasmic carboxy and amino termini are variable and probably function as regulatory domains, attachment sites for other proteins. Six potential sites for protein kinase C (PKC) phosphorylation were reported in human taurine transporter. PKC activation was reported to play some important roles in the regulation of taurine uptake in cultured cells (Kulanthaivel *et al.*, 1991). PMA is a tumor-promoter and can activate PKC (Blumberg,

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1988). It was known as an inhibitor for monocyte differentiation (Hass *et al.*, 1991).

Taurine has been found abundant in platelets, lymphocytes, retina and brain cells (Wright *et al.*, 1986). It reduced inflammation by modulating activities of macrophages (Banks *et al.*, 1992). Therefore uptake of taurine into macrophages is an important step in protecting tissue injuries. In this study, we attempted to find whether phorbol 12-myristate 13-acetate (PMA) and dexamethasone may influence taurine transport in macrophages.

Materials and Methods

Cell culture and taurine uptake

Taurine transportaion studies were performed on THP1 (human monocyte, ATCC TIB 202) and RAW 264.7 (mouse macrophage, ATCC TIB 71) cell lines that were obtained from American Type Culture Collection (Maryland, USA). Cells were cultured in DMEM medium containing 10% heat-inactivated fetal calf serum (FCS) and penicillin/streptomycin in 90 mm culture dishes during maintenancd and in 6-well plates for taurine uptake experiments. When the cells were confluent on the dish, the medium was removed from the monolayer culture and replaced with 2 ml of Na^+-containing 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 $MgSO_4$, 5 mM D-glucose, or 2 ml of Na^+-deficient buffer, which contained 140 mM sucrose instead of 140 mM NaCl. After stabilizing the cells for 2 h at 37℃, 2 μCi of [2-^{3}H(N)]-taurine (21.9 Ci/mmol, Dupont, NEN) were added to each culture and incubation continued for 40 min. The cells were washed thrice with ice-cold Na^+-deficient buffer and lyzed with each one ml of 0.01 mM NaOH and 1 mM EDTA. The lysate was mixed with 8 ml cocktail solution and was measured by liquid scintillation analyzer 2500TR (Hewlett-Packard). In most cases, the average counting efficiency was 34%.

Cell stimulation

RAW 264.7 cells were stimulated with IL-1 (100 ng/ml), IFN-γ (100 IU/ml), PMA (10 ng/ml) and dexamethasone (100 nM) for 6 h in 10% FCS-DMEM in 6-well plates. And the medium was replaced with Na^+ buffer and the cells were stabilized for 1 h. Then 20 μl (2 μCi) of [^{3}H]taurine in 2 ml buffer were added and cultured for 40 min. Uptake of [^{3}H]taurine was measured by a β-scintillation counter after the cells were lyzed. To see the effects of PMA treatment time for taurine uptake, the mouse macrophage cell line, RAW 264.7, was stimulated for various lengths of times with PMA (10 ng/ml) or PMA (10 ng/ml)+dexamethasone (100 nM) in 40 mm culture dishes in 10% DMEM medium. The medium was replaced with Na^+ buffer and stabilized for 10 min and then 2.5 μCi [^{3}H]taurine were added and incubated for 40 min. The cells were thoroughly washed with buffer and lyzed with 1 ml of 20 μM NaOH containing 1 mM EDTA for 1 h at room temperature. The lysates were used for measuring taurine uptake in this cell line.

For the pretreatments of Na^+-deficient buffer and cold-taurine in the human macrophage cells, THP1 cells were grown in RPMI 1640 medium containing 10% heat-inactivated FCS, penicillin/streptomycin, L-glutamine. The cells were washed with Na^+-containing or Na^+-deficient buffer and each 3×10^6 cells in one ml medium was stabilized in 24-well plates for 30 min at 37℃ in 7% CO_2 incubator. Added were 100 μl of 1 M cold taurine or PBS to cells and further incubated for 30 min. Then added were 2 μCi (20 μl) of [^{3}H]taurine to each well and incubated for 30 min. The cells were washed with ice-cold PBS three times and the cells were lyzed with 0.2 M NaOH. The lysates were used for measurement of the uptake of [^{3}H]taurine into the cells by adding 2 ml of the cocktail solution.

Results

Macrophage cells expressed taurine transporter

We measured the uptake of [^{3}H]taurine in human and mouse macrophage cell lines to examine whether macrophage cells express the taurine transporter on their cell membrane. When we compared the effects of Na^+-ion on taurine uptake in the RAW264.7 cell line, the cells in Na^+-deficient buffer did not transport much taurine into the cells, i.e., cells in Na^+-containing buffer and Na^+-deficient buffer transported [^{3}H]taurine 6,570,009 and 8,408 cpm, respectively (Table 1). Na^+-containing buffer induced 780-times higher taurine transport than Na^+-deficient buffer did in the mouse macrophage cells.

Similar experiments were conducted in the human macrophage cell line, THP1. In this cell line, the Na^+-containing buffer transported 33-times higher [^{3}H]taurine than the Na^+-deficient buffer (Table 2). In addition to this, when the THP1 cells were pretreated with 100 μM cold taurine for 30 min, [^{3}H]taurine uptake was almost similar to that of the Na^+-deficient buffer. These results showed that human and mouse macrophage cells expressed the taurine transporter on their cell membranes.

PKC stimulation blocked taurine uptake in macrophage

When the RAW264.7 cells were grown confluently on 6-well culture plates, they were treated with various concentrations of PMA, known as a PKC stimulator. After one hr, the medium was replaced with Na^+-containing buffer and stabilized for one hr and incubated with 2 μCi [^{3}H]taurine for 30 min. Low concentrations of PMA such as 0.1 or 1.0 ng/ml PMA did not affect [^{3}H]taurine uptake. However the concentration of 10 ng/ml PMA reduced [^{3}H]taurine uptake in the cell. The reduction of taurine uptake was gradual as the PMA concentration increased. At the concentration of 10 and 100 ng/ml PMA, [^{3}H]taurine uptake was 85% and 82% of that of the control, respectively (Table 3).

Dexamethasone abolished the PMA effect on taurine transportation

RAW264.7 cells were stimulated with 100 ng/ml IL-1β, 100 IU/ml IFN-γ, 10 ng/ml PMA or 100 nM dexamethasone for 6 h and cultured in the presence of [^{3}H]taurine for 40 mins. The single treatment or co-stimulation of the cells with IL-1β and IFN-γ did not affect [^{3}H]taurine uptake on the cell line in the presence of Na^+-containing buffer. However, addition of PMA to the culture system of IL-1β+IFN-γ reduced [^{3}H]taurine uptake to 82% of that of the control. Interestingly the addition of dexamethasone abolished the effect of PMA when the cell line was stimulated with IL-1β+IFN-γ+PMA+dexamethasone. These resultsshowed that inflammatory cytokines such as IL-1β or IFN-γ did not affect taurine transporter activity, but stimulation of protein kinase C with PMA reduced the activity of the transporter. When the cells were stimulated with PMA and dexamethasone simultaneously, dexamethasone blocked the signal transduction of PKC to the taurine transporter in the cytoplasm.

In the next experiment, the cell line was stimulated with 10 ng/ml PMA for 5 min, 30 min, 1 h and 3 h in the absence or presence of 100 nM dexamethasone. The results showed that 5 and 30 min stimulations with PMA could not recover the reduced [^{3}H] taurine transportation by the dexamethasone. At least one hr stimulation was required for the blocking of the signal transduction of PKC activity in this system (Table 5).

Discussion

In the present study, we demonstrated that macrophage cell lines express taurine transporter protein on their cell surface and phorbol-ester inhibits the activity of the transporter. Moreover this study is the first evidence for the regulating effect of dexamethasone on signal transduction of protein kinase C in taurine transporter activity in macrophage cells. The fact that human and mouse macrophage cell lines showed Na^+-ion dependent taurine uptake strongly indicates that these cells expressed taurine transporter on the cell surface. In addition, macrophage cells did not uptake [^{3}H]taurine when they were pretreated with cold taurine. Most of the taurine transporters of human, mouse, rat and dog showed Na^+ and Cl^- dependence. Therefore dependence on these ions during taurine uptake can be an indicator of the presence of taurine transporter. According to the study of taurine binding to its receptor by Wu (Wu *et al.*, 1990), it bound maximally at 30 min and reached a plateau at 40 min. There was almost no difference between 30 and 40 min incubations in the report. Therefore we treated cells with [^{3}H]taurine for 30 or 40 min to ensure maximal transportation. The discrepancy of the uptaken amounts of taurine into RAW264.7 cells could be clearly observed between the cells in Na^+-containing and Na^+-dificient

Table 1. Effect of Na^+ ion for taurine transportation in macrophage cell line, RAW264.7

Cell	[^{3}H]taurine transported (pmol)	
	Na^+ containing buffer	Na^+ deficient buffer
RAW	390.95± 27.99	0.51± 0.06

Cells were pulsed with [^{3}H]taurine for 40 min in Na^+-containing buffer (25 mM-HEPES/Tris (pH 7.5), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM D-glucose) or Na^+-deficient buffer which contained 140 mM sucrose instead of 140 mM NaCl. The lysate was mixed with 8 ml cocktail solution and was used in measuring β-count for three min.

Table 2. Pretrement of cold taurine prevented [^{3}H]taurine uptake in human promonocytic cell line, THP1

Buffer	Pretreatment	Treatment[a]	[^{3}H]Taurine transported (pmol)[b]
Na^+ def-buffer[c]	PBS	45 nM [^{3}H]Taurine	0.018± 0.001
Na^+ buffer	PBS	45 nM [^{3}H]Taurine	0.576± 0.075
Na^+ buffer	100 μM Taurine	45 nM [^{3}F]Taurine	0.013± 0.003

[a]THP1 cells were grown in RPMI 1640 medium containing 10% heat inactivated FCS, pen/str, L-glutamine. The cells were washed with Na^+-containing or Na^+-deficient buffer and each 3×10^6 cells in one ml medium stabilized in 24-well plate for 30 min at 37℃, 7% CO_2 incubator. Added 100 μl of 1 M cold taurine or PBS to cells and further incubated for 30 min. Then added 2 μCi (20 μl) of [^{3}H]taurine to each well and incubated for 30 min. The cells were washed with ice-cold PBS three times and lyzed the cells with 0.2 M NaOH.
[b]Mean± S.E. [c]Na^+ deficient buffer.

Table 3. [^{3}H]Taurine uptake was dose-dependently blocked by PMA stimulation in RAW 264.7

PMA (ng/ml, 1 h)	[^{3}H]Taurine (30 min)	[^{3}H]Taurine transported (pmol)	(%)
0.0 (PBS only)	45 nM	31.85± 1.96[a]	100
0.1	45 nM	31.81± 0.75	99 (NS)
1.0	45 nM	30.63± 4.34	98 (NS)
10	45 nM	27.13± 5.28	85 (NS)
100	45 nM	25.93± 1.26	82 ($p<0.05$)
1,000	45 nM	24.23± 1.04	77 ($p<0.05$)

Cells were cultured and stimulated in 6-well plate.
[a]Values are mean± S.E. NS: Not significant.

buffers by pulsing for 40 min (Table 1). Stoichiometrically, the ratios of Na^+ : taurine and Cl^- : taurine during taurine uptake were 2 : 1 and 1 : 1, respectively (Tiruppathi *et al.*, 1992; Ramamoorthy *et al.*, 1993). RAW 264.7 cells activated by interferon-gamma showed inhibition of the synthesis of nitric oxide (NO) and tumor necrosis factor by treatment with taurine chloramine (Park *et al.*, 1993).

PMA is known as a PKC activator. Upon activation, PKC moves from the cytosol to the intracellular membrane. As shown in Table 3, the activity of taurine transporter was dose-dependently inhibited by PMA. The inhibition was observed only above 10 ng/ml PMA, which is a typical concentration for exertion of activity. PKC was found to inhibit the activity of taurine transporter in various tissues such as a placental choriocarcinoma cell line (Kulanthaivel *et al.*, 1991), renal epithelial cell (Jones *et al.*, 1991) and a colon carcinoma cell line (Brandsch *et al.*, 1993). Most of the taurine transporter molecules from various species have 12 transmembrane domains and their sizes are about 70 kDa (Jhiang *et al.*, 1993). The long cytoplasmic carboxy and amino termini of the transporter are variable and probably function as regulatory domains, attachment sites for other proteins. Six potential sites for PKC phosphorylation were reported in human taurine transporter (Jhiang *et al.*, 1993). PKC activation was reported to play some important roles in the regulation of taurine uptake in cultured cells (Kulanthaivel *et al.*, 1991). The involvement of PKC in taurine transporter activity was recently further confirmed by using staurosporine, an inhibitor of PKC activity (Han *et al.*, 1995a). It is known that diacylglycerol (DAG) is an intracellular PKC stimulator, whereas PMA is a plant component and may not exhibit the same function as DAG does in the RAW264.7 cells (Morgan, 1989). Some of the metabolic intermediates could lead to inhibition or recovery of the transporter activities during six h incubation (Table 4). However this possibility could be excluded by the fact that PMA inhibited activities of the transporter in the case of 30 min treatment (Table 5). The activity of taurine transporter of human placental cells was inhibited even in 15 min and maximally inhibited in 60 min by PMA. Their result is very similar with that of our result (Kulanthaivel *et al.*, 1991). It is still unknown whether PKC may act directly or indirectly on taurine transporter in the macrophage. Furthermore it may need to be clarified which isotype of the PKC is involved in the inhibition of the activity of the taurine transporter in macrophage cells. The mechanisms of the taurine transporter for taurine uptake and release in macrophages should be studied in detail. And the signal pathways for the regulation of the activity of the transporter by various stimulators are also required to be elucidated.

Table 4. Dexamethasone recovers reduced taurine uptake by PMA in RAW cell line

Stimulation	[^{3}H]Taurine transported (pmol)	(%)
PBS	28.39± 0.11[a]	100
IL-1β	27.08± 0.61	95 (NS)
IFN-γ	26.94± 0.14	93 (NS)
IL-1β+IFN-γ	29.20± 0.53	102 (NS)
IL-1β+IFN-γ+PMA	24.71± 0.30	82 ($p<0.01$)
IL-1β+IFN-γ+PMA+Dex	30.17± 0.11	107 (NS)

RAW 264.7 cells were stimulated with stimulators for 6 h in 10% FCS-DMEM in 6-well plate. And the medium was replaced with Na^+ buffer and cells were stabilized for 1 h. Then added 2 μCi (20 μl) [^{3}H]Taurine in 2 ml buffer and cultured for 40 min. Uptaken [^{3}H]Taurine was measured by β-scintillation counter after the cells were lyzed. The final concentrations were as follows: IL-1β (100 ng/ml), IFN-γ (100 IU/ml), PMA (10 ng/ml) and dexamethasone (100 nM).
[a]Data are shown in mean± S.E. NS: Not significant.

During the onset of inflammation in mammals, macrophages and lymphocytes are activated and secret various cytokines such as IL-1 and IFN-γ. The secreted IL-1 stimulates hypothalamus and pituitary gland and results in release of cortisol (Goulding and Guyre, 1992 and 1993). To see whether PMA, dexamethasone and those acute inflammatory cytokines such as IL-1β and IFN-γ could influence the taurine transporter activity, we examined these reagents in our system. Our results showed that both IL-1β and IFN-γ did not inhibit the transporter in RAW264.7 and that combination treatment of the cells with IL-1β, IFN-γ and PMA inhibited the transporter activity. Addition of dexamethasone to this system led to recovery of the activity of the transporter (Table 4). However dexamethasone itself did not seem to increase the activity of taurine transporter in

Table 5. Dexamethasone recovers reduced taurine uptake induced by PKC stimulation after one h

Stimulation	[^{3}H]Taurine transported (pmol)			
	Without dexamethasone	(%)	With dexamethasone	(%)
PBS	15.73± 0.42[a]	100	14.10± 0.58[a]	90 (NS)
PMA (5 min)	15.68± 0.30	99 (NS)	13.39± 0.29	85 ($p<0.01$)
PMA (30 min)	13.51± 0.36	86 ($p<0.05$)	12.56± 0.20	80 ($p<0.01$)
PMA (1 h)	11.89± 0.43	75 ($p<0.01$)	15.31± 0.60	97 (NS)
PMA (3 h)	13.87± 0.33	88 ($p<0.05$)	ND	

Mouse macrophage cell line, RAW 264.7, were stimulated for various times with PMA (10 ng/ml) or PMA (10 ng/ml)+dexamethasone (100 nM) in 40 mm culture dish in 10% DMEM medium. The medium was replaced with Na^+ buffer and stabilized for 10 min and then added 2.5 μCi [^{3}H]Taurine for 40 min. The cells were lyzed with 1 ml of 20 μM NaOH containing 1 mM EDTA for 1 h at room temperature.

[a]The data are expressed as mean± S.E. ND : Not determined, NS : Not significant.

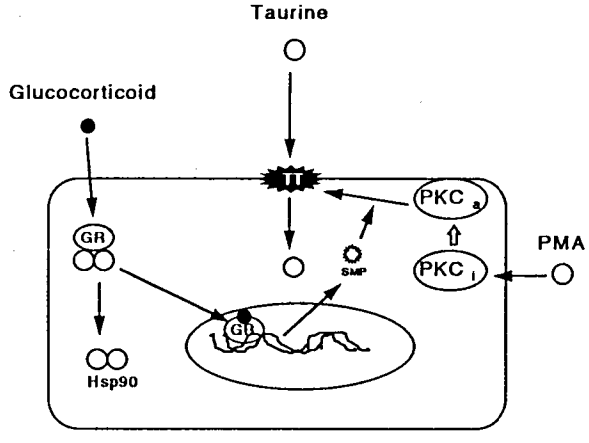

Fig. 1. Hypothetical signal pathway on the taurine transporter activity in macrophage cells. Activated PKC inhibits the activity of the taurine transporter. Glucocorticoid hormone may block the signal pathway of the PKC to the transporter. Abbreviations are GR : glucocorticoid hormone; hsp 90 : heat shock protein 90; PKCi : inactive protein kinase C; PKCa : active PKC; PMA : phorbol myristate acetate; SMP : second messenger protein; TT : taurine transporter.

the macrophage cells (Table 5). Dexamethasone is one of the glucocorticoid hormones and its receptor is present in the cytoplasm. The complex of dexamethasone and its receptor moves to the nucleus and binds to DNA resulting in modulation of some protein expression. The activity of the transporter that was inhibited by PKC might be recovered by dexamethasone through induced protein(s) as shown in Fig. 1. The second messenger protein(s) induced by dexamethasone may block the signal pathway of activiated PKC. This can be verified by the time-course recovery experiment that dexamethasone could lead to recovery of [^{3}H]taurine uptake, but required one h in PMA treated RAW cells (Table 5). The cells that were treated with dexamethasone for short time periods such as 5 or 30 min failed to show recovery of the activity of taurine transporter. The effects of dexamethasone can appear either in a short time or in a delayed fashion. A rapid response by the glucocorticoid hormone can be mediated by the constituent protein(s) in the cell. A delayed response, however, can be mediated by synthesized second messenger protein(s) (Peers *et al.*, 1993).

Injection of the antibody that is directed to the highly conserved intracellular segment between transmembrane domains III and IV inhibited taurine transporter activity of rat renal epithelial cells (Han *et al.*, 1995b). Our results that PMA-treatment led to partial blocking of the transporter activities (Table 3) might be due to the alteration of the intracellular domain(s) of the transporter by direct or indirect modulation of the transporter. The transporter regulation by PMA occurred slowly, since the inhibition was not observed within 5 min (Table 5).

The fact that dexamethasone led to recovery of taurine uptake in the PMA-stimulated cells suggests that macrophage cells may require higher taurine concentration in a stressed state, since the glucocorticoid hormone could be secreted into the blood stream by the activation of the hypothalamo-pituitary-adrenocortical (HPA) axis due to stress or injury (Goulding and Guyre, 1992 and 1993).

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