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Biochimica et Biophysica Acta 1235 (1995) 351–360

BBA Biochimica et Biophysica Acta

Substrate-specific regulation of the taurine transporter in human placental choriocarcinoma cells (JAR)

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Received 1 September 1994; revised 21 November 1994; accepted 16 December 1994

Abstract

Exposure of the JAR human placental choriocarcinoma cells to taurine leads to a marked decrease in the activity of the taurine transporter in these cells. The ability to induce this adaptive response is not unique to taurine but is shared by other substrates of the transporter as well. Compounds such as betaine and α-aminoisobutyric acid which are not substrates for the transporter do not produce this effect. The change in the taurine transporter activity induced by taurine exposure is however unique to the taurine transporter because the activities of many other transport systems remain unaffected under these conditions. The adaptive regulation is not associated with any change in the dependence of the transporter activity on Na^+ and Cl^-, in the Na^+/Cl^-/taurine stoichiometry and in the affinities of the transporter for Na^+ and Cl^-. The decrease in the transporter activity caused by taurine exposure is due to a decrease in the maximal velocity of the transporter, and to a lesser extent, in the substrate affinity of the transporter. The decrease in the transporter activity observed in intact cells is demonstrable in plasma membrane vesicles after isolation from control and taurine-exposed cells. Cycloheximide and actinomycin D block the adaptive response in intact cells to a significant extent, but not completely. Northern blot analysis of mRNA from control and taurine-exposed cells shows that taurine exposure causes a significant decrease in the steady state levels of the taurine transporter mRNA. It is concluded that the activity of the taurine transporter in JAR cells is subject to substrate-specific adaptive regulation and that transcriptional as well as posttranscriptional events are involved in this regulatory process.

Keywords: Taurine transporter; Adaptive response; mRNA level; Protein synthesis; RNA synthesis; Choriocarcinoma cell; (Placenta)

1. Introduction

Human placenta expresses an active transport system for taurine and other β-amino acids in the maternal-facing brush border membrane [1,2]. This taurine transporter has been recently cloned and characterized [3]. The transporter is believed to be obligatorily involved in the transplacental transfer of taurine from mother to fetus. Since the human fetus has no ability to synthesize taurine endogenously and yet is known to accumulate very high levels of this amino acid in tissues, the function of the transporter assumes great importance as being the sole mechanism available for the fetus to meet its taurine requirements. The JAR human placental choriocarcinoma cell line which has been shown to express the taurine transporter [4] has proved to be very useful in the investigation of the regulatory aspects of the transporter. The function of the transporter is regulated by protein kinase C [4] and calmodulin-dependent processes [5], but is unaffected by cAMP [6,7].

There is evidence that certain amino acid transport systems are subject to adaptive regulation. In this type of regulation, cells can maintain their physiological and biochemical states by appropriately modulating the rate of transport across the plasma membrane when subjected to changing amino acid concentrations. The mechanism involved in the process may be either up- or down-regulation of the plasma membrane transport in response to changing states in amino acid availability. Examples of amino acid transport systems which are regulated in this manner include systems A [8,9], L [10], X_{AG}^- [11], and N^m [12]. Animal studies have shown that the taurine transporter

Abbreviations: SDS, sodium dodecyl sulfate; EGTA, (ethylene bis(oxyethylenenitrilo))tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetate; AIB, α-aminoisobutyric acid; GES, guanidinoethanesulfonic acid.

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SSDI 0005-2736(95)00006-2

expressed in the kidney is also subject to adaptive regulation [13–15]. Recently, the modulation of the taurine transporter activity by the availability of taurine has been demonstrated in cultured cell lines of renal origin [16,17].

The ability of the kidneys to conserve taurine in women undergoes significant changes during pregnancy and lactation [18]. There is a need for the mother to conserve this amino acid during these reproductive stages because of the increased demands. Fetal tissues accumulate large quantities of taurine which has to be obtained solely from the mother because fetal tissues, including the placenta, have no or only negligible capacity to synthesize the amino acid [19,20]. Human milk contains high levels of taurine and the secretion of taurine in milk causes increased demands for this amino acid in lactating women. Accordingly, the ability of the kidney to reabsorb taurine increases significantly both during pregnancy and lactation as a physiological response to meet the needs of the developing fetus and the suckling infant for this amino acid. Whether the placenta is also capable of regulating its taurine transport capacity in response to taurine availability in the mother has not been investigated. Since transplacental transfer of taurine is an obligatory step in satisfying the fetal demands for this amino acid, it is very likely that the placenta offers an additional site of regulation to ensure optimal taurine nutrition in the developing fetus. The present study was undertaken to investigate the adaptive regulation of the human placental taurine transporter using the JAR human placental choriocarcinoma cell line as an experimental model.

2. Materials and methods

2.1. Materials

The JAR human placental choriocarcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). RPMI-1640 medium, penicillin and streptomycin were purchased from Life Technologies (Gaithersburg, MD, USA). Fetal bovine serum was from Atlanta Biologicals (Atlanta, GA, USA). Amino acids, insulin, prostaglandin E_1, apotransferrin, hydrocortisone, thyroxine, cycloheximide and actinomycin D were obtained from Sigma (St. Louis, MO, USA). Guanidinoethanesulfonic acid was a gift from Prof. T. Hoshi, University of Shizuoka, Shizuoka, Japan.

[2-^{3}H]Taurine (spec. radioactivity, 21.9 Ci/mmol), β-[3-^{3}H]alanine (spec. radioactivity, 92.6 Ci/mmol), L-[4,5-^{3}H]leucine (spec. radioactivity, 60 Ci/mmol) L-[3-^{3}H]alanine (spec. radioactivity, 76.9 Ci/mmol), and 5-[1,2-^{3}H]hydroxytryptamine (serotonin) (spec. radioactivity, 26.4 Ci/mmol) were purchased from DuPont-New England Nuclear (Boston, MA, USA). [5,6-^{3}H]Uridine (spec. radioactivity, 30 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA).

2.2. Methods

Culture of JAR human placental choriocarcinoma cells

The JAR cells were cultured in 75 cm^2 culture flasks using RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml) and streptomycin (100 μg/ml). Cultures were maintained at 37° C in an atmosphere of 95% air/5% CO_2 till they reached confluence. Subcultures were started from the cells released by trypsin treatment. The cells were seeded in 35 mm Petri dishes at a density of $1.5 \cdot 10^6$ cells/dish and allowed to grow for 24 h in the culture medium which contained 5% fetal bovine serum. Following this, the medium was removed and the cells were allowed to grow for an additional 24 h (for varying time in one experiment) in a hormonally defined medium in the absence of serum. The defined medium [21] was made up of RPMI-1640 medium containing penicillin (100 units/ml), streptomycin (100 μg/ml), insulin (5 μg/ml), apotransferrin (5 μg/ml), prostaglandin E_1 ($2.5 \cdot 10^{-5}$ M), hydrocortisone ($5 \cdot 10^{-8}$ M) and thyroxine ($5 \cdot 10^{-12}$ M). Stock solutions of prostaglandin E_1, hydrocortisone and thyroxine were prepared in dimethylsulfoxide. The final concentration of dimethylsulfoxide in the defined medium was 0.125%. The amino acid substrates used to study the adaptive response were added to the defined medium. Thus, the cells were exposed to these substrates for 24 h prior to uptake measurement. When the involvement of RNA and protein synthesis in the adaptive response was studied, actinomycin D (0.02 μg/ml) or cycloheximide (25 μM), dissolved in dimethylsulfoxide, was added to the defined medium. In these experiments, the final concentration of dimethylsulfoxide in the medium was 0.335%.

Uptake measurement

Confluent monolayer cultures were used for uptake measurements. All the steps in uptake measurements were carried out at room temperature. Briefly, the medium was aspirated and the monolayer was washed once with the uptake buffer. Fresh uptake buffer (1 ml) containing the radiolabeled compounds (taurine, β-alanine, α-alanine, leucine or serotonin) was added to the dish and incubated for 3 min. Uptake was terminated by aspirating the medium and subsequently washing the monolayer thrice with the uptake buffer. The cells were then lysed with 1 ml of 0.2 M NaOH/1% SDS and the contents were transferred to a counting vial to determine radioactivity. The composition of the uptake buffer in most experiments was 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 glucose.

Measurement of leucine and uridine incorporation into acid-insoluble fraction

This was done as described previously [7]. Radiolabeled leucine (1 μCi/dish) or uridine (0.25 μCi/dish) was added directly to the culture medium bathing the monolay-

ers and incubation continued for 2 h at 37° C. Following this, the medium was removed by aspiration and the monolayers were washed four times with 10% trichloroacetic acid and once with an ether/ethanol (1:3, v/v) mixture. The dishes were allowed to dry and the contents were dissolved in 1 ml of 0.2 M NaOH/1% SDS and used for determination of radioactivity.

A similar procedure was used to determine the fraction of leucine or α-alanine that was incorporated into cellular proteins under the incubation conditions employed for uptake measurements.

Preparation of plasma membrane vesicles

Isolation of plasma membrane vesicles from JAR cells was done as described previously [22]. The JAR cells were grown in 225 cm^2 culture flasks for 24 h in the presence of 5% fetal bovine serum. Following this, the medium was replaced with the hormonally defined medium with or without taurine and the cells were grown for an additional 24 h. At the end of this treatment, the culture flasks were kept on ice for 15 min and the medium was removed by aspiration. The cells were washed twice with ice-cold phosphate-buffered saline. The cells were then suspended in ice-cold 10 mM Tris-HCl buffer (pH 7.5) containing 2 mM EGTA and 10 mM KF (25 ml/flask) and homogenized in an Ultra-Turrax tissuemizer. Stock solution (1 M) of $MgCl_2$ was added to the homogenate to give a final concentration of 12 mM $MgCl_2$ and the mixture was stirred at 4° C for 10 min. The suspension was centrifuged at $3000 \times g$ for 15 min and the resulting supernatant was again centrifuged at $60000 \times g$ for 30 min. The final pellet was rinsed with and suspended in 10 mM Hepes/Tris buffer (pH 7.5) containing 280 mM mannitol, 2 mM EGTA and 10 mM KF. The activity of alkaline phosphatase, a marker enzyme for the plasma membrane, measured with *p*-nitrophenyl phosphate as the substrate [23] was enriched 8 ± 2-fold in the final membrane preparations compared to the starting cell homogenate.

Uptake measurement in membrane vesicles

This was done as detailed previously [1,24]. Briefly, 40 μl of membrane vesicle suspension (200 μg membrane protein) was mixed with 160 μl of uptake buffer containing radiolabeled taurine or serotonin and incubated for desired time at room temperature. Initial uptake rate and equilibrium uptake were determined using 15 s and 6 h incubation periods respectively. Uptake was terminated by the addition of 3 ml of ice-cold stop buffer followed by rapid filtration through a Millipore filter (DAWP type, 0.65 μm pore size). The filter retaining the membrane vesicles was washed with 3×5 ml of the stop buffer and the radioactivity associated with the filter was determined by liquid scintillation spectrometry. The uptake buffer was 10 mM Hepes/Tris (pH 7.5) containing 140 mM NaCl or 140 mM KCl. The stop buffer was 5 mM Hepes/Tris (pH 7.5) containing 160 mM KCl.

Protein determination

Protein was assayed for each experiment by using duplicate dishes cultured concurrently and under conditions identical with those dishes used for uptake measurements. Deionized water (1 ml) was added to each dish. The dishes were frozen and thawed twice, after which the contents were suspended to form a homogenate by using a 1 ml syringe and 25-gauge needle. Protein concentration of the homogenate was determined by the method of Lowry et al. [25].

Isolation of poly(A)$^+$ RNA and Northern blot analysis

Poly(A)$^+$ RNA was isolated from JAR cells which were treated for 24 h with or without 250 μM taurine in a defined medium. This was achieved using FastTrack mRNA isolation kit (Invitrogen, San Diego, CA, USA). Poly(A)$^+$ RNA was size-fractionated on a denaturing formaldehyde-agarose gel and transferred to an activated nylon membrane (Hybond N$^+$, Amersham, Arlington Heights, IL, USA). Following overnight prehybridization of the membrane at 42° C in 50% formamide, $10 \times$ Denhardt's solution, $5 \times$ SSPE ($1 \times$ SSPE = 0.15 M NaCl, 32 mM NaH_2PO_4, 1 mM Na_2 EDTA, pH 7.4), 2% SDS, and 100 μg/ml freshly denatured and sheared salmon DNA, hybridization was initiated by the addition of the cDNA probe and continued at 42° C for 24 h. The blot was washed twice at 22° C in $2 \times$ SSPE and 0.1% SDS, followed by a 1-h wash at 65° C in $0.1 \times$ SSPE and 0.1% SDS, and exposed to autoradiographic film. The same blot was used for the Northern analysis of the taurine transporter mRNA and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. This was done by sequential hybridization, stripping, and rehybridization with respective cDNA probes. Sizes of hybridizing RNAs are based on parallel electrophoresis of RNA standards. Quantification of transcript signals in autoradiograms was done with a dual wavelength scanner (Shimadzu, model CS-9000).

The taurine transporter cDNA probe was a 705 bp fragment of the coding region of the human placental taurine transporter cDNA [3]. The GAPDH cDNA probe was a 0.78 kb fragment obtained from a human GAPDH clone (ATCC, Rockville, MD, USA) by digestion with *Pst*I and *Xba*I. The cDNA probes were gel purified and radiolabeled with [α-^{32}P]dCTP (Amersham, Arlington Heights, IL, USA) by random priming. The labeled cDNAs were purified from free nucleotides by size-exclusion chromatography on a Sephadex G-50 column.

Statistics and data analysis

Each experiment was repeated 2–3 times with replicate dishes and the results are given as means $\pm$ S.E. Kinetic analyses were carried out using the computer software *Fig. P.* version 6.0 (BioSoft, Cambridge, UK). The kinetic parameters, K_t and V_{max}, were calculated by linear regression analysis (Eadie-Hofstee method).

3. Results

3.1. Effects of exposure of JAR cells to taurine on the activity of the taurine transporter

The JAR cells were exposed to varying concentrations (0–250 μM) of taurine in a hormonally defined medium for 24 h. Following this treatment, the activity of the taurine transporter in these cells was measured by determining the uptake rate of radiolabeled taurine. Exposure of the cells to taurine resulted in a marked decrease in the taurine transporter activity (Fig. 1). This adaptive response was dose-dependent. The decrease was 70–75% when the concentration of taurine during the exposure period was 250 μM.

Fig. 2 describes the time-dependence of the adaptive response. In this experiment, cells were exposed to 250 μM taurine for varying time, following which the medium was removed and the activity of the taurine transporter was determined by measuring the uptake of radiolabeled taurine. The transporter activity was found to decrease as the time of exposure of the cells to unlabeled taurine increased. The maximal decrease ($\sim$ 80%) was observed with an exposure time of 12 h.

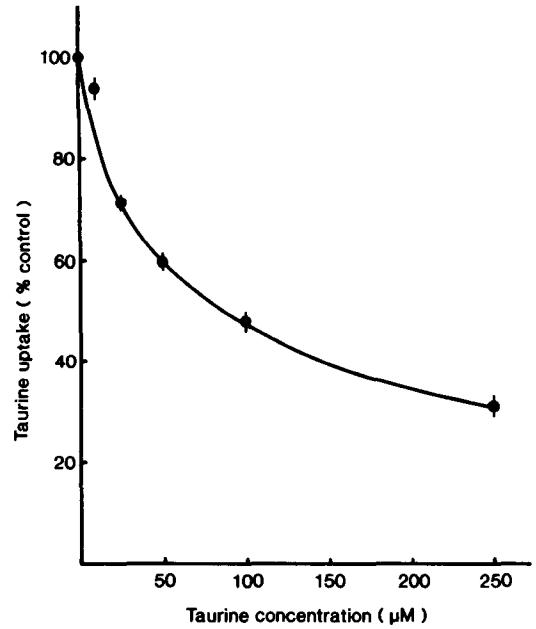

Fig. 1. Dose–response relationship for the inhibition of the taurine transporter by treatment of cells with taurine. Confluent monolayer cultures of JAR cells were treated with varying concentrations of taurine (0–250 μM) in a hormonally defined medium for 24 h at 37° C. After the treatment, the medium was removed and the monolayers were washed twice with the uptake buffer. The activity of the taurine transporter was determined by measuring the uptake of [^{3}H]taurine (30 nM) using an incubation time of 3 min. Uptake in control cells which were not treated with taurine was 1.51 $\pm$ 0.09 pmol/3 min per mg of protein and this value was taken as 100%. The data represent means $\pm$ S.E. from four determinations.

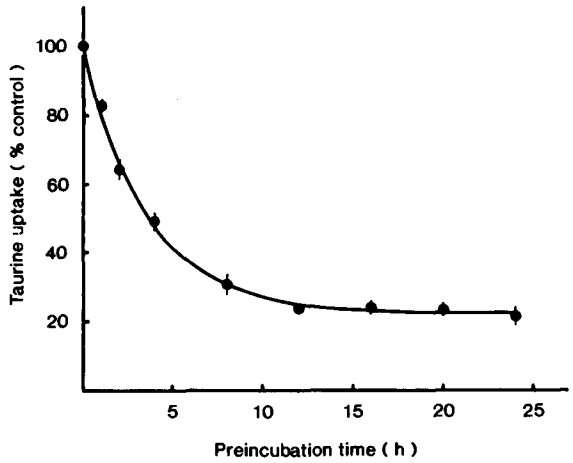

Fig. 2. Time-dependence of the inhibition of the taurine transporter by treatment of the cells with taurine. Confluent monolayer cultures of JAR cells were treated with 250 μM taurine for varying time periods in a hormonally defined medium at 37° C. After the treatment, the medium was removed and the monolayers were washed twice with the uptake buffer. The activity of the taurine transporter was determined by measuring the uptake of [^{3}H]taurine (30 nM) using an incubation time of 3 min. Uptake in control cells which were not treated with taurine was 1.30 $\pm$ 0.04 pmol/3 min per mg of protein and this value was taken as 100%. The data represent means $\pm$ S.E. from four determinations.

3.2. Specificity of the adaptive response

To determine which amino acids were capable of regulating the taurine transporter function, the cells were exposed to different amino acids for 24 h in a defined medium following which the activity of the taurine transporter was measured (Table 1). Taurine, hypotaurine, β-alanine, and guanidinoethanesulfonic acid were able to

Table 1
Specificity of the substrate-dependent regulation of the taurine transporter activity in JAR cells

Test substrate	Uptake (pmol/3 min per mg of protein)	
	taurine	β-alanine
None	1.03 $\pm$ 0.04 (100)	0.159 $\pm$ 0.009 (100)
Taurine	0.19 $\pm$ 0.02 (19)	0.035 $\pm$ 0.003 (22)
Hypotaurine	0.21 $\pm$ 0.04 (20)	0.033 $\pm$ 0.002 (21)
β-Alanine	0.39 $\pm$ 0.01 (38)	0.055 $\pm$ 0.003 (35)
GES	0.47 $\pm$ 0.04 (46)	0.069 $\pm$ 0.006 (43)
AIB	1.04 $\pm$ 0.05 (101)	0.157 $\pm$ 0.009 (99)
Betaine	1.15 $\pm$ 0.05 (112)	0.179 $\pm$ 0.005 (113)

Confluent monolayer cultures of JAR cells were treated with or without different test substrates (250 μM) in a hormonally defined culture medium for 24 h at 37° C. After the treatment, the medium was removed and the monolayers were washed with the uptake buffer. The activity of the taurine transporter was determined by measuring the uptake of [^{3}H]taurine (30 nM) or β-[^{3}H]alanine (15 nM). The uptake buffer was 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 glucose. The time of incubation for uptake measurements was 3 min. The values in parentheses are percent of uptake in control cells treated in the absence of test substrates. The data represent means $\pm$ S.E. from four determinations.

decrease the activity of the transporter and the order of potency was taurine = hypotaurine > β-alanine > guanidinoethanesulfonic acid. All four amino acids are substrates for the taurine transporter. The decrease in the activity of the taurine transporter was evident whether the activity was measured as taurine uptake or β-alanine uptake. The inhibitory effects of taurine, hypotaurine, β-alanine, and guanidinoethanesulfonic acid as well as the order of their potency were identical for the uptake of both taurine and β-alanine. In contrast, AIB and betaine which are not substrates for the taurine transporter were unable to regulate the taurine transporter activity. Thus, the adaptive response of the taurine transporter in the JAR cells was specific towards the substrates of the transporter.

We also studied the specificity of the response with respect to the transport system involved (Table 2). The JAR cells were treated in the presence or absence of taurine (250 μM) for 24 h, following which the activities of four different transport systems were measured. The activity of the taurine transporter measured as the uptake of taurine decreased in an adaptive response to exposure to taurine. In contrast, the activities of the serotonin transporter (serotonin uptake) and the amino acid transport systems ASC (α-alanine uptake) and L (leucine uptake) remained unaltered. In these experiments, uptake of α-alanine and leucine was measured with a 3 min incubation. Under these conditions, the measured values represented predominantly transport. The fraction of the amino acid that was incorporated into proteins was found to be 1.3 ± 0.2% for α-alanine and 18.6 ± 0.3% for leucine. However, since the value was a significant fraction of the measured uptake in the case of leucine, we have repeated the leucine uptake measurements in control and in taurine-exposed cells with a 1 min incubation. With this incubation period, the fraction of leucine that was incorporated into proteins was only 2.0 ± 0.2%. There was again no difference in leucine uptake between control and taurine-exposed cells, the values being 0.25 ± 0.01 and 0.23 ± 0.01 pmol/min per mg of protein respectively. Thus, the adaptive response to taurine exposure is specific for the taurine transporter.

Table 2
Influence of culturing the JAR cells in the presence of taurine on the activities of different transport systems

Transporter substrate	Uptake (pmol/3 min per mg of protein)	
	control	taurine-treated
Taurine	1.35 ± 0.09 (100)	0.32 ± 0.05 (24)
Serotonin	0.46 ± 0.02 (100)	0.44 ± 0.02 (96)
α-Alanine	1.16 ± 0.08 (100)	1.14 ± 0.01 (98)
Leucine	0.44 ± 0.03 (100)	0.45 ± 0.06 (103)

Confluent monolayer cultures of JAR cells were treated with or without 250 μM taurine in a hormonally defined culture medium for 24 h at 37° C. After the treatment, the medium was removed and the monolayers were washed twice with the uptake buffer. The activities of the taurine transporter, serotonin transporter, and amino acid transport systems ASC and L were determined by measuring the uptake of taurine (30 nM), serotonin (75 nM), α-alanine (10 nM) and leucine (5 nM), respectively. The time of incubation for uptake measurements was 3 min. The values in parentheses are percent of uptake in respective control cells treated in the absence of taurine. The data represent means ± S.E. from four determinations

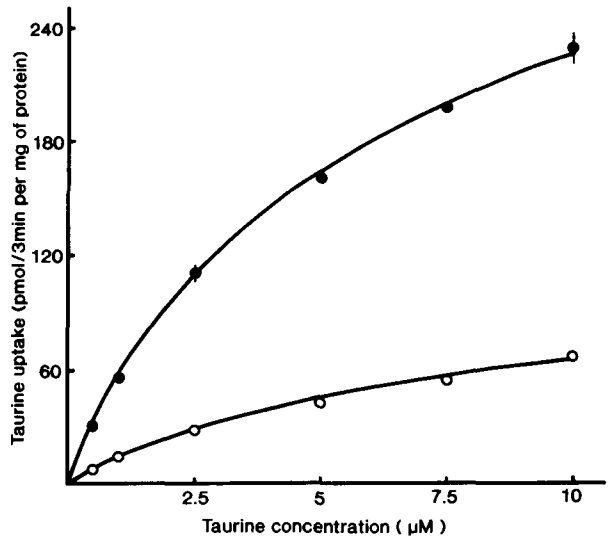

Fig. 3. Kinetic analysis of taurine uptake in control and taurine-treated cells. Confluent monolayer cultures of JAR cells were treated with (○) or without (●) taurine (250 μM) in a hormonally defined medium for 24 h at 37° C. After the treatment, the medium was removed and the monolayers were washed twice with the uptake buffer. The NaCl-dependent uptake of taurine was measured over a concentration range of 0.5–10 μM, using an incubation time of 3 min. Concentration of [^{3}H]taurine was kept at 0.05 μM and the final concentration of taurine was adjusted by adding appropriate amounts of unlabeled taurine. Uptake of radioactivity measured in the presence of an excess amount of unlabeled taurine (500 μM) was used to determine the nonmediated uptake at each concentration of taurine. This value was subtracted from total uptake to calculate the mediated uptake. The data represent means ± S.E. from four determinations.

3.3. Kinetics of taurine uptake in control and taurine-exposed cells

The dependence of the uptake rate on taurine concentration was investigated in control and in taurine-exposed cells (Fig. 3). In both cases, the uptake rate was hyperbolically related to taurine concentration, suggesting participation of a saturable transport system. Eadie-Hofstee plots (uptake rate/taurine concentration versus uptake rate) were linear ($r^2 > 0.99$) in control as well as in taurine-exposed cells (data not shown). The kinetic constants, K_t (Michaelis-Menten constant) and V_{max} (maximal velocity), in control cells were 4.9 ± 0.2 μM and 329 ± 10 pmol/3 min per mg of protein, respectively. The corresponding values in taurine-exposed cells were 6.7 ± 0.4 μM and 104 ± 4 pmol/3 min per mg of protein. Thus, the taurine-induced adaptive inhibition of the activity of the taurine transporter is associated with a decrease in the affinity of the transporter for this substrate and also with a decrease in the maximal velocity of the transporter. The effect on the

maximal velocity was however much greater than the effect on the affinity.

3.4. Na^+- and Cl^--kinetics of the taurine transporter in control and taurine-treated cells

The activity of the taurine transporter in JAR cells is dependent on Na^+ as well as Cl^- [4]. This characteristic is also true in the case of the transporter expressed in the brush border membrane of the normal placenta [1,26]. Stoichiometric analysis has shown that 2 Na^+ and 1 Cl^- are involved in the transport of one taurine molecule in placental brush border membrane vesicles [3,26,27]. The Na^+/Cl^-/taurine stoichiometry has not been determined for the JAR cell taurine transporter. Since exposure of JAR cells leads to a decrease in the taurine transporter activity, it was of interest to determine whether this adaptive change was associated with alterations in the Na^+ – and Cl^--dependence and kinetics. Therefore, we investigated the dependence of the taurine uptake rate on Na^+ concentration in the presence of a fixed concentration of Cl^- and on Cl^- concentration in the presence of a fixed concentration of Na^+ in control cells and in taurine-exposed cells. The relationship between uptake rate and Na^+ concentration was found to be sigmoidal in control and in taurine-exposed cells (data not shown), indicating involvement of more than one Na^+ ion in the transport of one taurine molecule. The experimental data were fit into the Hill-type equation

$$v = \frac{V_M \cdot [Na^+]^n}{K_{0.5}^n + [Na^+]^n}$$

where v is the uptake rate, V_M is the uptake rate at infinite concentration of Na^+, $K_{0.5}$ is the concentration of Na^+ necessary to give an uptake rate equal to $0.5 \times V_M$ and n is the number of Na^+ ions involved per transport cycle. Nonlinear regression analysis of the data according to this equation gave a value of 2.1 ± 0.2 for n and 68 ± 4 mM for $K_{0.5}$ in control cells. The corresponding values in taurine-exposed cells were 1.8 ± 0.1 and 65 ± 3 mM. These results show that the number of Na^+ ions involved in the transport cycle and the affinity of the transporter for Na^+ (as indicated by $K_{0.5}$) remain unaltered after exposure to taurine.

Similar experiments on the dependence of the taurine transporter activity on Cl^- concentration revealed that the relationship was hyperbolic in control and in taurine-treated cells (data not shown). This suggests that one Cl^- ion is involved in the transport of one taurine molecule in both cases. The value for $K_{0.5}$ for Cl^- was 51 ± 14 mM in control cells and 36 ± 11 mM in taurine-treated cells. Thus, the number of Cl^- ions involved in the transport process and the affinity of the transporter for Cl^- were not affected by taurine exposure.

The procedure used here to determine the Na^+/taurine and Cl^-/taurine coupling ratios is called 'activation' method [28]. This approach has been widely used to determine stoichiometric relationships for various ion-coupled transport systems. However, it has to be mentioned that what is measured in this approach is the dose-response relationship for the ability of the coupling ions to activate the transport of the substrate. The actual transport of the ions which is coupled to the transport of the substrate is not measured. Since the validity of this method depends on the mechanism involved in the activation process, this can be considered at best only an indirect approach to determine the stoichiometry.

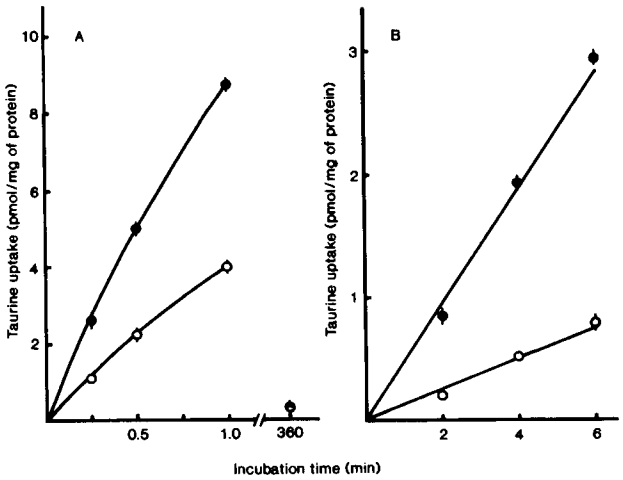

Fig. 4. Time-course of taurine uptake in plasma membrane vesicles (A) and in intact cells (B). Confluent cultures of JAR cells were treated with (○) or without (●) 250 μM taurine in a hormonally defined culture medium for 24 h at 37° C. After treatment, the medium was removed by aspiration and the monolayers were rinsed twice with a taurine-free medium. The cells were then either used directly for uptake measurements or used for preparation of plasma membrane vesicles. Uptake in membrane vesicles was measured in the presence of an inwardly directed NaCl gradient at a taurine concentration of 0.2 μM. Uptake in intact cells was measured in the presence of NaCl in the medium at a taurine concentration of 30 nM. The data represent means ± S.E. from four determinations.

3.5. Uptake of taurine and serotonin in plasma membrane vesicles prepared from control and taurine-exposed cells

To determine whether the substrate-specific adaptive regulation of the taurine transporter activity observed in intact cells can be shown at the level of the plasma membrane, control cells and taurine-exposed cells were lysed and used for isolation of plasma membrane vesicles. Uptake of taurine was measured in these membrane preparations in the presence of an inwardly directed NaCl gradient (Fig. 4A). The uptake was linear at least up to 30 s in membrane vesicles prepared from control as well as taurine-exposed cells. The initial uptake rate measured with a 15 s incubation was 55% less in membrane vesicles from taurine-exposed cells than from control cells. This decrease in the uptake was not due to alterations in the

vesicle volume as evident from the findings that there was no difference in the equilibrium uptake (6 h incubation) between these two membrane preparations. The uptake measured with shorter incubations was many-fold greater than the uptake measured with a 6 h incubation in both membrane preparations, demonstrating a transient uphill transport of taurine in response to the transmembrane NaCl gradient. For comparison, the time-course of taurine uptake in intact cells is given in Fig. 4B. The uptake was linear in control as well as in taurine-exposed cells for at least upto 6 min. The uptake was 75% less in control cells compared to taurine-exposed cells at all time periods measured.

A possible explanation for the observed changes in the taurine transport activity in plasma membrane vesicles isolated from control versus taurine-exposed cells may be that the plasma membrane permeability for Na^+ and Cl^- increases as a result of exposure of the cells to taurine. We tested this possibility by measuring the activity of the serotonin transporter in these membrane vesicles under identical conditions. The taurine transporter as well as the serotonin transporter belong to the Na^+- and Cl^--coupled transporter family. Any change in the Na^+- and Cl^--permeabilities of the plasma membrane is expected to affect the activity of the serotonin transporter as well. The uptake of serotonin (0.2 μM) measured in plasma membrane vesicles derived from control cells was 1.11 ± 0.03 pmol/15 s per mg of protein. The corresponding value in plasma membrane vesicles derived from taurine-exposed cells was 1.21 ± 0.08 pmol/15 s per mg of protein. The lack of effect of taurine exposure on the activity of the serotonin transporter suggests that the membrane permeabilities for Na^+ and Cl^- remain unaltered during exposure of the cells to taurine. Therefore, the observed decrease in taurine transport in membrane vesicles from taurine-exposed cells versus control cells cannot be due to alterations in the Na^+- and Cl^--permeability characteristics of the plasma membrane resulting from exposure to taurine.

3.6. Effects of inhibitors of protein and RNA synthesis on the taurine-induced adaptive regulation of the taurine transporter

To evaluate the role of synthesis of the taurine transporter protein in the adaptive regulation of the transporter activity, the cells were treated with or without taurine in the presence or absence of cycloheximide, an inhibitor of protein synthesis, and actinomycin D, an inhibitor of RNA synthesis. Uptake of taurine was subsequently measured in these cells (Table 3). In the absence of inhibitors, exposure to taurine caused a 72% decrease in the transporter activity. This decrease could be blocked to a significant extent, but not completely, when the cells were treated with taurine in the presence of inhibitors of RNA and protein synthesis. The taurine-induced decrease in the transporter activity was 38% in the presence of cycloheximide and 56% in the presence of actinomycin D. That cycloheximide was able to block protein synthesis under the experimental conditions employed was clearly evident from a 97% decrease as the result of cycloheximide treatment in the incorporation of leucine into acid-insoluble fraction (data not shown). Similarly, actinomycin D was very effective in blocking RNA synthesis and, as a result, in protein synthesis. Treatment of the cells with actinomycin D decreased the incorporation of uridine into acid-insoluble fraction by 97%. The actinomycin D-induced decrease in incorporation of leucine was 90% (data not shown).

Table 3
Effects of cycloheximide and actinomycin D on substrate-dependent regulation of the taurine transporter activity in JAR cells

Treatment	Uptake of taurine (pmol/3 min per cm^2)
None	0.251 ± 0.003 (100)
Taurine	0.069 ± 0.003 (28)
Cycloheximide	0.156 ± 0.001 (100)
Cycloheximide + taurine	0.096 ± 0.003 (62)
Actinomycin D	0.135 ± 0.003 (100)
Actinomycin D + taurine	0.059 ± 0.001 (44)

Confluent monolayer cultures of JAR cells were treated with or without taurine (250 μM) in the absence or presence of cycloheximide (25 μM) or actinomycin D (0.02 μg/ml) in a hormonally defined culture medium for 16 h at 37° C. After the treatment, the medium was removed and the monolayers were washed twice with the uptake buffer. The activity of the taurine transporter was determined by measuring the uptake of [^{3}H]taurine (30 nM) by using an incubation period of 3 min. The values in parentheses are percent of uptake in respective control cells treated in the absence of taurine. The data represent means ± S.E. from six determinations

3.7. Steady state levels of taurine transporter mRNA in control and taurine-exposed cells

To determine whether alterations in the steady state levels of the taurine transporter mRNA play a role in the taurine-induced adaptive regulation of the taurine transporter activity, we estimated the levels of the taurine transporter mRNA in control and taurine-exposed cells. These cells possess multiple forms of the transporter mRNA and a 6.9 kb transcript is the most predominant among these forms. The levels of this transcript decreased by 67% in taurine-exposed cells compared to control cells (Fig. 5). In these experiments, the levels of the GAPDH mRNA were also analyzed as an internal control for RNA loading and transfer efficiency. The taurine transporter mRNA/GAPDH mRNA ratio was 1.59 in control cells. This ratio decreased by 65% to 0.56 in taurine-exposed cells. In these experiments, cells which were exposed to AIB served as negative control. Exposure of the cells to AIB did not alter the taurine transport activity and accordingly there was no noticeable change in the steady state levels of the 6.9 kb transcript. These experiments were repeated three times and the decrease in the taurine trans-

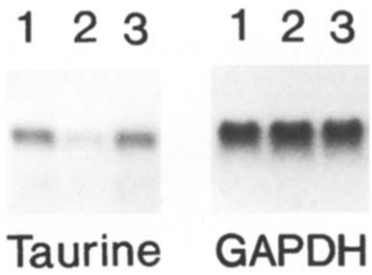

Fig. 5. Northern blot analysis. Confluent monolayer cultures of JAR cells were treated with 250 μM taurine, 250 μM AIB or without these animo acids in a hormonally defined medium for 24 h at 37° C. After the treatment, poly(A)$^+$ RNA was isolated from the cells. The size-fractionated mRNA (lane 1, control cells; lane 2, taurine-exposed cells; lane 3, AIB-exposed cells) was probed with the human placental taurine transporter cDNA. The lower panel shows the corresponding Northern blot analysis of GAPDH mRNA. The same membrane blot which was used for the analysis of the taurine transporter mRNA was used to analyze the GAPDH mRNA. This was done by stripping the membrane off radioactivity and rehybridizing with the human GAPDH cDNA probe.

porter mRNA levels as the result of exposure of the cells to taurine ranged between 35 and 65% with a mean value of 51%.

4. Discussion

The present study shows that exposure of the JAR placental choriocarcinoma cells to taurine down regulates the activity of the taurine transporter. This effect is very specific for the substrates of the transporter. This absolute substrate specificity is interesting because a strict correlation between substrate specificity and ability to induce adaptive response has not been observed in the case of the amino acid transport systems L [10], A [29,30] and X_{AG}^- [11]. Though the adaptive regulation of the taurine transporter has been studied previously in renal cell lines [16,17], the substrate specificity of the phenomenon has not been investigated. The results of the present study showing that the ability to induce adaptive response in the activity of the taurine transporter is not unique to taurine alone but is in fact shared by the other substrates of the transporter are of importance because these results are relevant to the understanding of the cellular mechanisms which underlie this adaptive regulation. A similar strict correlation between substrate specificity and ability to induce adaptive response has been recently observed in the case of the amino acid transport system N^m [12]. In addition, as observed in the present study, the adaptive response induced by the substrates of system N^m is specific to this transport system with no change in the activities of other transport systems.

The present study provides detailed information on the kinetic characteristics of the taurine transporter in control cells and in taurine-exposed cells. In both cases, the basic properties of the transporter such as dependence of the transporter activity on Na^+ and Cl^- and the Na^+/Cl^-/taurine stoichiometry for the transport process remain the same. Similarly, there is no detectable change in the affinities of the transporter for Na^+ and Cl^-. The decrease in the transporter activity caused by exposure to taurine is primarily associated with a decrease in the maximal velocity and, to a lesser extent, with a decrease in the affinity of the transporter for taurine. This change in the affinity as a contributory factor in adaptive regulation is unique to the taurine transporter because the other transport systems which are subject to adaptive regulation such as the amino acid transport systems L, A, X_{AG}^-, and N^m exhibit no change in K_t in association with adaptive response [8–12].

The experiments with the inhibitors of RNA and protein synthesis show that the increase in the activity of the taurine transporter as a result of exposure of the JAR cells to the taurine-free culture medium is significantly blunted by cycloheximide and actinomycin D. This suggests that synthesis of new taurine transporter protein (or other possible regulatory proteins) may be involved in the process. This is supported by the increase in the V_{max} as well as in the steady state levels of the taurine transporter mRNA as a result of taurine deprivation. However, synthesis of new transporter protein alone is insufficient to explain the observed effects. The activity of the taurine transporter increases 3 to 4-fold in taurine-deprived cells compared to taurine-exposed cells. In contrast, this process is accompanied by only a 2-fold increase in the taurine transporter mRNA levels. Therefore, it is likely that other processes such as synthesis of regulatory proteins and/or posttranslational modification of the taurine transporter protein are involved in the adaptive regulation of the taurine transporter.

To our knowledge, there has been only one report which describes the changes in transporter-specific mRNA levels in association with adaptive regulation of an amino acid transporter system [11]. In this case however, the increase in the activity of the transport system X_{AG}^- as a result of amino acid starvation is accompanied by an initial decrease in the transporter mRNA levels followed by a later restoration to control levels. It therefore appears that posttranscriptional events are principally responsible for adaptive regulation in the case of system X_{AG}^-. In contrast, taurine deprivation in JAR cells leads to a significant increase in the transporter mRNA levels which is at least partly responsible for the increase in the transporter activity observed under these conditions.

When there is a decrease in the activity of a transporter in intact cells as a result of exposure to the substrates of the transporter, it can be argued that the decrease in the activity is due to the higher intracellular concentration of the substrates in the substrate-exposed cells compared to control cells. However, the results of the present study with purified plasma membrane vesicles do not support this argument. The change in the taurine transporter activity observed in intact cells as a consequence of exposure to

taurine is observable even at the level of plasma membrane vesicles. The changes seen in the taurine transporter activity in membrane vesicles are not due to taurine-induced alterations in Na^+- and Cl^--permeability characteristics of the plasma membrane because the activity of the serotonin transporter, which is also a Na^+- and Cl^--coupled transport system, remains unaltered under similar conditions. Interestingly, the change in the activity of the transporter in plasma membrane vesicles is significantly less than the change in intact cells. The activity in taurine-exposed cells is 25–30% of the activity in control cells when the activity is measured in intact cells. However, when plasma membrane vesicles purified from these cells are used to determine the transporter activity, the corresponding value is only 45%. If posttranslational modification of the transporter protein such as phosphorylation/dephosphorylation is at least partly responsible for the adaptive regulation, these covalent changes in the protein may be reversed to some extent during the membrane isolation procedure. This might explain the observed quantitative difference between intact cells and plasma membrane vesicles in the decline of the taurine transporter activity as a result of exposure of the cells to taurine. The time-dependence of the adaptive response showing that the taurine transport activity is decreased significantly even when the cells are exposed to taurine for a period as short as 1 h supports the notion that acute effects such as posttranslational modification may be involved in this process. However, possible contribution of trans-inhibition of the taurine transporter by intracellular taurine to the observed difference between intact cells and plasma membrane vesicles cannot be entirely ruled out.

The cellular mechanisms which underlie the adaptive regulation of the activity of the taurine transporter in JAR cells are not known. In the case of the amino acid transport system L, it appears that the concentration of charged $tRNA^{Leu}$ molecules serves as the signal for inducing adaptive regulation in response to leucine availability [31,32]. The substrates of the taurine transporter, in contrast, are not incorporated into proteins and there are no specific tRNAs for these amino acids. It has been suggested that accumulation of taurine inside the cells during exposure to taurine might modulate protein phosphorylation and thereby affect taurine transport [17]. In fact, there is evidence that taurine is indeed capable of inhibiting phosphorylation of specific proteins in a variety of tissues [33]. But the present study indicates that the ability to induce adaptive response in the activity of the taurine transporter is by no means unique to taurine. This characteristic is shared by the other substrates of the transporter as well. It is however not known whether these other substrates also have the ability to inhibit protein phosphorylation, but it seems very unlikely. It therefore appears that some other cellular mechanisms must be involved in this phenomenon. The JAR cell taurine transporter is highly active and its concentrative capacity is remarkable. When these cells are exposed to the substrates of the taurine transporter for 24 h, these substrates are expected to be accumulated inside the cell to very high levels resulting in a significant change in the osmolality of the intracellular milieu. This change, with the accompanying alterations in the cell volume, might trigger cellular responses including changes in intracellular Ca^{2+} levels [34] which might play a role in the modulation of transcriptional as well as posttranscriptional processes.

The relevance of the findings presented here with cultured cells to the process of taurine transport from mother to fetus across the normal placenta is not known. It is likely that the placenta also possesses the ability to regulate the function of the taurine transporter in response to the concentration of taurine in the maternal circulation. This would enable the placenta to maintain optimal taurine nutrition in the developing fetus. In view of the accumulating evidence for a crucial role of taurine in development [33,35], it is obvious that regulation of the placental transport of this amino acid is of physiologic significance.

Acknowledgements

This work was supported by the National Institutes of Health Grant HD 24451 and by a grant from Taisho Pharmaceutical Company. The authors acknowledge the expert secretarial assistance of Joyce Hobson.

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