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$Na^+ + Cl^-$-gradient-driven, high-affinity, uphill transport of taurine in human placental brush-border membrane vesicles

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Uptake of taurine in human placental brush-border membrane vesicles was greatly stimulated in the presence of an inwardly-directed $Na^+ + Cl^-$-gradient and uphill transport of taurine could be demonstrated under these conditions. Na^+ as well as Cl^- were obligatory for this uptake and both ion gradients could energize the uphill transport. This $Na^+ + Cl^-$-gradient-dependent taurine uptake was stimulated by an inside-negative membrane potential, demonstrating the electrogenicity of the process. The uptake system was highly specific for β-amino acids and the K_m of the system for taurine was 6.5 ± 0.4 μM.

Taurine; Active transport; NaCl gradient; (Placenta, Brush-border, Human)

1. INTRODUCTION

Taurine, 2-aminoethanesulfonic acid, is a β-amino acid which is present in many tissues of man and other animal species in millimolar concentrations [1,2]. This amino acid appears to play an important role in neurotransmission, and in the function of the retina, heart and muscle [1–3]. Taurine can be synthesized to some extent endogenously from methionine and cysteine in adult animals, including man, but the biosynthetic capacity during fetal development is negligible [4]. However, compared to adult tissues, fetal tissues contain higher concentrations of taurine [5], indicating that the placenta possesses an efficient mechanism to transfer this amino acid from the mother to the fetus. In spite of this obligatory role of the placental transfer in supplying the fetal demands of this important amino acid, virtually nothing is known about this process. We report here, for the first time, on the characteristics of taurine transport in brush-border membrane vesicles purified from normal human term placentas.

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2. EXPERIMENTAL

Brush-border membrane vesicles were isolated from normal human term placentas by an Mg^{2+}-aggregation method as described [6]. The purity of the membrane preparations was routinely assessed by measuring the activity of marker enzymes, alkaline phosphatase and 5'-nucleotidase [7,8]. In most of the experiments, the membrane vesicles were preloaded with 10 mM Hepes/Tris buffer, pH 7.5, containing 300 mM mannitol. Uptake measurements were made by a rapid filtration technique [9,10] using Millipore filters (DAWP type; pore size, 0.65 μm).

Uptake measurements were routinely done in duplicate or triplicate and the replicate values were always within ± 5% of the mean value. All data are expressed as mean ± SD from 3–6 determinations.

[2-^{3}H(N)]Taurine (spec. act. 20.9 Ci/mmol) was obtained from New England Nuclear, Boston, MA.

3. RESULTS AND DISCUSSION

We first examined the effects of inwardly-

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directed NaCl and KCl gradients on the uptake of taurine in human placental brush-border membrane vesicles. Fig.1 shows that taurine uptake was negligible in the presence of a KCl gradient, but it was markedly stimulated by the presence of a NaCl gradient. The initial rates of uptake measured with a 15 s incubation were 80–120-fold greater in the presence of a NaCl gradient than in the presence of a KCl gradient. The intravesicular accumulation of taurine at 5 min was greater than the accumulation at 60 min (1.25-fold) or at 180 min (5.0-fold), indicating transient uphill transport of this amino acid into the membrane vesicles.

It has recently been reported by Chesney et al. [11] and by Turner [12] that the β-amino acid transport system in renal brush-border membranes is coupled to both Na^+ and Cl^-. A similar Cl^--dependency has also been demonstrated for taurine transport in synaptosomes [13] and liver [14]. Therefore, we investigated the specificity of monovalent cations and anions in stimulating taurine uptake in human placental brush-border membrane vesicles (table 1). In the presence of an inwardly-directed Na^+ gradient, taurine uptake was found to be markedly anion-dependent, the uptake being the highest in the presence of Cl^- and almost nonexistent in the presence of F^-. Among the anions tested, the ability to stimulate the Na^+-dependent taurine uptake was in the following order: $Cl^- > SCN^- > I^- > NO_3^- > F^-$. However, when there was no Na^+, taurine uptake was negligible even in the presence of an inwardly-directed Cl^- gradient. These data demonstrate that Na^+ is obligatory for the transport of taurine in placental brush-border membrane vesicles and this Na^+-dependent taurine uptake is markedly dependent on Cl^-.

We further investigated the role of Cl^- in the Na^+-dependent taurine transport to see if the Cl^--dependent stimulation of taurine uptake was due to the presence of an inwardly-directed Cl^- gradient or the presence of Cl^- per se. The data in fig.2 show that the initial uptake rates of taurine were higher in the presence of Cl^- than in the presence of F^-, demonstrating the obligatory role of Cl^- in the uptake process. However, when the uptake rates measured in the presence of an inwardly-directed Cl^- gradient ($[Cl^-]_o$ = 120 mM; $[Cl^-]_i$ = 0) were compared with the uptake rates measured in the presence of Cl^-, but in the absence

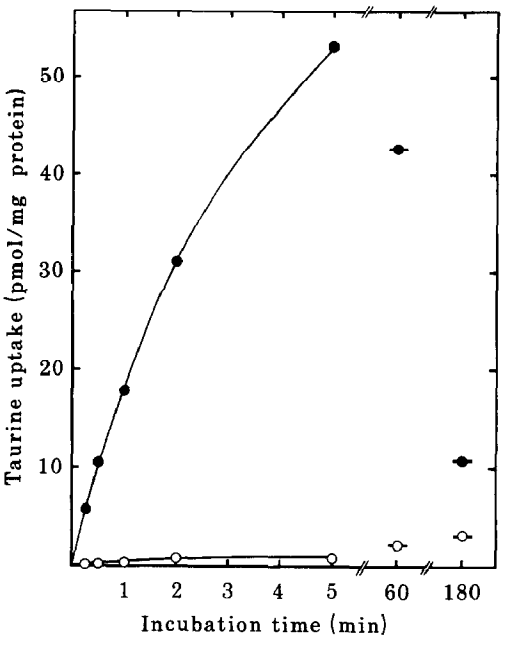

Fig.1. Time course of taurine uptake in the presence of NaCl or KCl in the uptake medium. The membrane vesicles were preloaded with 300 mM mannitol, buffered with 10 mM Hepes/Tris, pH 7.5. Taurine uptake was measured in 10 mM Hepes/Tris buffer, pH 7.5, in the presence of either 120 mM NaCl or 120 mM KCl. The final concentration of taurine was 1 μM. (●—●) NaCl; (○—○) KCl.

of a Cl^- gradient ($[Cl^-]_o = [Cl^-]_i$ = 120 mM), it was evident that the uptake was stimulated by a Cl^- gradient rather than by Cl per se.

The electrogenic nature of this $Na^+ + Cl^-$-gradient-driven transport of taurine was then examined. Fig.3 shows that a valinomycin-induced K^+-diffusion potential which was inside-negative accelerated the initial uptake rates of taurine by about 3-fold compared to the uptake rates measured in short-circuited membrane vesicles. These results demonstrate that the $Na^+ + Cl^-$-gradient-dependent taurine uptake in human placental brush-border membrane vesicles is electrogenic in nature, resulting in a transfer of positive charge across the membrane.

The substrate specificity of the carrier system was investigated by studying the effects of a variety of unlabeled amino acids on the uptake of labeled taurine. Human placental brush-border membranes have been shown to possess three distinct carrier systems for neutral α-amino acids, system

Table 1

Effects of monovalent cations and anions on taurine uptake

Inorganic salts	Taurine uptake	
	pmol/mg protein per 15 s	%
NaCl	5.80 ± 0.47	100
NaI	1.66 ± 0.09	29
$NaNO_3$	1.47 ± 0.10	25
NaSCN	3.16 ± 0.16	54
NaF	0.31 ± 0.03	5
KCl	0.14 ± 0.02	2
LiCl	0.12 ± 0.02	2
RbCl	0.11 ± 0.04	2

The membrane vesicles were preloaded with 300 mM mannitol, buffered with 10 mM Hepes/Tris, pH 7.5. The initial uptake rates were measured with 15 s incubations in the presence of NaCl or other inorganic salts (120 mM). The final concentration of taurine was 1 μM. The results represent mean ± SD from three determinations

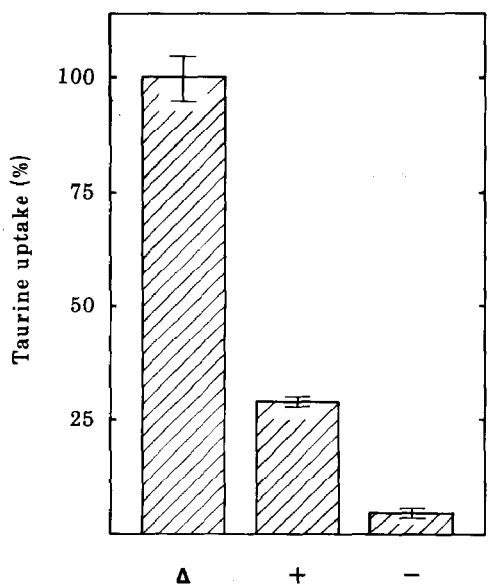

Fig.2. Effects of Cl^- on taurine uptake. The membrane vesicles were preloaded with a 10 mM Hepes/Tris buffer, pH 7.5, containing either 300 mM mannitol or 150 mM KCl. The uptake buffer was 10 mM Hepes/Tris, pH 7.5, containing either 150 mM NaCl or 150 mM NaF. The initial uptake rates of taurine were measured with 15 s incubations in the presence of a Cl^- gradient ($[Cl^-]_o$ = 120 mM; $[Cl^-]_i$ = 0), in the presence of Cl^- but in the absence of a gradient ($[Cl^-]_o$ = $[Cl^-]_i$ = 120 mM) and in the absence of Cl^-. The final concentration of taurine was 1 μM. The data are given as a percent of taurine uptake in the presence of a Cl^- gradient (7.13 ± 0.37 pmol/mg protein per 15 s = 100). The results are mean ± SD from three determinations. (Δ) Cl^- gradient; (+) Cl^- present, but no gradient; (−) Cl^- absent.

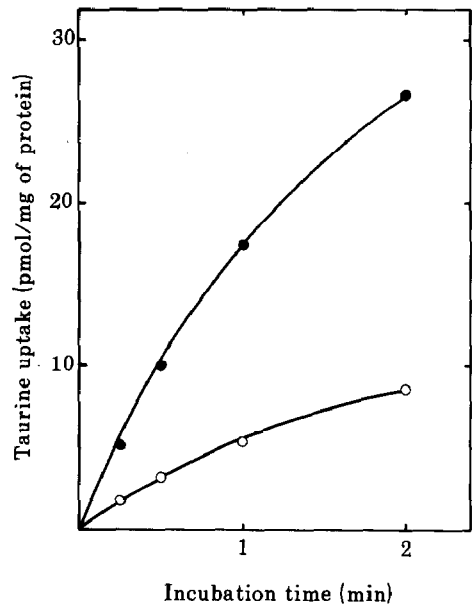

Fig.3. Effect of an inside-negative membrane potential on taurine uptake. The membrane vesicles were preloaded with a 10 mM Hepes/Tris buffer, pH 7.5, containing 300 mM mannitol and 100 mM K-gluconate. The uptake buffer was either 10 mM Hepes/Tris, pH 7.5, containing 150 mM NaCl and 100 mM K-gluconate (control) or 10 mM Hepes/Tris, pH 7.5, containing 150 mM NaCl and 200 mM mannitol. Valinomycin (final conc. 5 μM) was present in both cases. The final concentration of taurine was 1 μM. (○—○) Control; (●—●) membrane potential.

A, system ASC and system ℓ [8,15]. In order to see if any of these transport systems is responsible for the transport of taurine, a neutral β-amino acid, in these membranes, we examined the effects of alanine and serine (system ASC-specific amino acids), threonine, proline and α-aminoisobutyric acid (system A-specific amino acids) and leucine and 2-amino-2-norbornanecarboxylic acid (system ℓ-specific amino acids) on taurine uptake. At a concentration of 100 μM, all these amino acids failed to inhibit the uptake of 1 μM taurine (table 2). These results demonstrate that the amino acid transport systems A, ASC and ℓ do not catalyze the transport of taurine in human placental brush-border membrane vesicles. Transport systems highly specific for β-amino acids have been described in various tissues such as kidney, brain, heart, liver and retina [1,2]. To find out whether a similar system is responsible for taurine uptake in placental brush-border membrane vesicles, we

studied the ability of β-amino acids, taurine, hypotaurine and β-alanine, to inhibit the uptake of radiolabeled taurine. Table 2 shows that these amino acids, at a concentration of 100 μM, inhibited the uptake of 1 μM radiolabeled taurine by 75–90%. Therefore, we conclude that human placental brush-border membranes possess a transport system highly specific for β-amino acids. However, it appears that this transport system may interact with small α-amino acids such as alanine, serine and α-aminoisobutyric acid but with a very low affinity because, at higher concentrations, these amino acids were found to have significant inhibitory effects on taurine uptake.

The dependence of the uptake rate on substrate concentration was then investigated for taurine uptake in these membrane vesicles. Initial uptake rates were measured with a 15 s incubation (the uptake rate was found to be linear at least up to 30 s) and the concentration of taurine was varied over the range 0.2–10 μM. The results are given in fig.4 as an Eadie-Hofstee plot (initial uptake rate/substrate concentration vs initial uptake rate). The linear plot ($r = -0.99$) shows that the taurine uptake system is saturable and that it obeys Michaelis-Menten kinetics describing a single system. The K_m for taurine was 6.5 ± 0.4 μM and the maximal velocity was 27.7 ± 1.0 pmol/mg protein per 15 s.

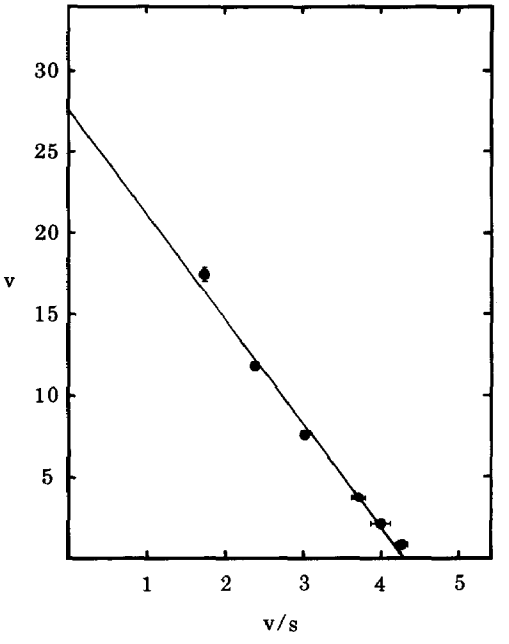

Fig.4. Kinetics of taurine uptake. The membrane vesicles were preloaded with 300 mM mannitol, buffered with 10 mM Hepes/Tris, pH 7.5. The initial uptake rates of taurine were measured with 15 s incubations in the presence of 120 mM NaCl. Concentration of taurine was varied between 0.2–10 μM. The results are given as an Eadie-Hofstee plot (mean ± SD; n = 6). Where not shown, the variation was within the symbol. s, taurine concentration (μM); v, initial uptake rate (pmol/mg protein per 15 s).

Table 2

Effects of amino acids on taurine uptake

Inhibitors	Taurine uptake			
	A		B	
	pmol/mg protein per 15 s	%	pmol/mg protein per 15 s	%
None	5.26 ± 0.27	100	5.26 ± 0.27	100
Taurine	0.49 ± 0.01	9	0.20 ± 0.07	4
Hypotaurine	0.48 ± 0.02	9	0.18 ± 0.06	3
β-Alanine	1.39 ± 0.03	26	0.33 ± 0.02	6
Alanine	4.42 ± 0.21	84	3.27 ± 0.08	62
Serine	4.75 ± 0.18	90	3.50 ± 0.13	67
Threonine	5.31 ± 0.21	101	4.57 ± 0.29	87
Proline	5.23 ± 0.10	99	4.31 ± 0.15	82
α-Aminoisobutyric acid	4.92 ± 0.29	94	3.83 ± 0.13	73
Leucine	5.43 ± 0.24	103	5.02 ± 0.22	95
2-Amino-2-norbornane carboxylic acid	5.66 ± 0.21	108	5.13 ± 0.15	98

The membrane vesicles were preloaded with 300 mM mannitol, buffered with 10 mM Hepes/Tris, pH 7.5. The initial uptake rates of taurine were measured with 15 s incubations in the presence of 120 mM NaCl. The final concentration of taurine was 1 μM. The concentration of amino acids was either 0.1 mM (A) or 1 mM (B). The results represent mean ± SD from three determinations

The transport systems driven by a H^+ gradient alone [6,16] or a Na^+ gradient alone [17] have been shown to reach equilibrium in placental brush-border membrane vesicles at a much shorter time than the taurine transport system. The energization of placental taurine transport by an $Na^+ + Cl^-$-gradient rather than an Na^+ gradient alone may have a physiological significance. Because of the limited ability of the fetus to synthesize taurine endogenously, this amino acid has to be transported from the mother to the fetus across the placenta. Taurine appears to play a crucial role in the development and maintenance of

normal function of many important organs such as brain, heart and eye, and therefore optimal transfer of this amino acid across the placenta is vital to the growth and development of the fetus. Since many amino acids are transported across the placenta by Na^+-dependent mechanisms, one might expect competition between taurine and other amino acids for the Na^+ gradient as the driving force. If taurine transport were to be dependent on a Na^+ gradient alone, such a competition could result in suboptimal transfer of this important amino acid across the placenta. The energization of the placental β-amino acid transport system by an $Na^+ + Cl^-$-gradient rather than by an Na^+ gradient alone would significantly reduce this competition and ensure optimal transfer of taurine across the placenta under physiological conditions.

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REFERENCES

[1] Chesney, R.W. (1985) Adv. Pediatr. 32, 1–42.
[2] Wright, C.E., Tallan, H.H. and Lin, Y.Y. (1986) Annu. Rev. Biochem. 55, 427–453.
[3] Huxtable, R.J. (1981) in: The Role of Peptides and Amino Acids as Neurotransmitters (Lombardini, J.B. and Kenny, A.D. eds) pp.53–97, Liss, New York.
[4] Sturman, J.A., Rassin, D.K. and Gaull, G.E. (1977) Pediatr. Res. 11, 28–33.
[5] Sturman, J.A., Rassin, D.K. and Gaull, G.E. (1977) Life Sci. 21, 1–22.
[6] Balkovetz, D.F., Leibach, F.H., Mahesh, V.B., Devoe, L.D., Cragoe, E.J., jr and Ganapathy, V. (1986) Am. J. Physiol. 251, C852–C860.
[7] Ganapathy, M.E., Mahesh, V.B., Devoe, L.D., Leibach, F.H. and Ganapathy, V. (1985) Am. J. Obstet. Gynecol. 153, 83–86.
[8] Ganapathy, M.E., Leibach, F.H., Mahesh, V.B., Howard, J.C., Devoe, L.D. and Ganapathy, V. (1986) Biochem. J. 238, 201–208.
[9] Ganapathy, V., Mendicino, J.F. and Leibach, F.H. (1981) J. Biol. Chem. 256, 118–124.
[10] Miyamoto, Y., Ganapathy, V. and Leibach, F.H. (1986) J. Biol. Chem. 261, 16133–16140.
[11] Chesney, R.W., Gusowski, N., Dabbagh, S., Theissen, M., Padilla, M. and Diehl, A. (1985) Biochim. Biophys. Acta 812, 702–712.
[12] Turner, R.J. (1986) J. Biol. Chem. 261, 16060–16066.
[13] Meiners, B.A., Speth, R.C., Bresolin, N., Huxtable, R.J. and Yamamura, H.I. (1980) Fed. Proc. 39, 2695–2700.
[14] Bucuvalas, J.C., Goodrich, A.L. and Suchy, F.J. (1987) Am. J. Physiol. 253, G351–G358.
[15] Yudilevich, D.L. and Sweiry, J.H. (1985) Biochim. Biophys. Acta 822, 169–201.
[16] Ganapathy, M.E., Leibach, F.H., Mahesh, V.B., Devoe, L.D. and Ganapathy, V. (1986) Biochem. Pharmacol. 35, 3989–3994.
[17] Ganapathy, V., Ganapathy, M.E., Tiruppathi, C., Miyamoto, Y., Mahesh, V.B. and Leibach, F.H. (1988) Biochem. J. 249, 179–184.