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The role of chloride in taurine transport across the human placental brush-border membrane

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Taurine, a sulfated β-amino acid, is conditionally essential during development. A maternal supply of taurine is necessary for normal fetal growth and neurologic development, suggesting the importance of efficient placental transfer. Uptake by the brush-border membrane (BBM) in several other tissues has been shown to be via a selective Na^+-dependent carrier mechanism which also has a specific anion requirement. Using BBM vesicles purified from the human placenta, we have confirmed the presence of Na^+-dependent, carrier-mediated taurine transport with an apparent K_m of 4.00 ± 0.22 μM and a V_{max} of 11.72–0.36 pmol mg^{-1} protein 20 s^{-1}. Anion dependence was examined under voltage-clamped conditions, in order to minimize the contribution of membrane potential to transport. Uptake was significantly reduced when anions such as thiocyanate, gluconate, or nitrate were substituted for Cl^-. In addition, a Cl^--gradient alone (under Na^+-equilibrated conditions) could energize uphill transport as evidenced by accelerated uptake (3.13 ± 0.8 pmol mg^{-1} protein 20 s^{-1}) and an overshoot compared to Na^+, Cl^- equilibrated conditions (0.60 ± 0.06 pmol mg^{-1} protein 20 s^{-1}). A Cl^--gradient (Na^+-equilibrated) also stimulated uptake of [^{3}H]taurine against its concentration gradient. Analysis of uptake in the presence of varying concentrations of external Cl^- suggested that 1 Cl^- ion is involved in Na^+/taurine cotransport. We conclude that Na^+-dependent taurine uptake in the placental BBM has a selective anion requirement for optimum transport. This process is electrogenic and involves a stoichiometry of 2:1:1 for Na^+/Cl^-/taurine symport.

Introduction

Taurine, 2-aminoethane sulfonic acid, is a sulfur-containing β-amino acid and the relatively inert end-product of methionine and cysteine metabolism. Recent interest has focused on the role of taurine as a conditionally essential amino acid in the developing fetus and newborn. Taurine accumulates rapidly during gestation, particularly in the fetal brain and liver, and these relatively high levels are maintained in the immediate postnatal period [1–4]. Many developing animals, including man, have a limited capacity for endogenous biosynthesis of taurine due largely to decreased activity of the rate-limiting enzyme cysteine sulfinic acid decarboxylase [4,5]. These observations would imply an important role for placental transfer of taurine during gestation.

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Transport of taurine across other epithelial membranes has been well characterized and involves a relatively high-affinity, Na^+-dependent, carrier-mediated process which is specific for β-amino acids [6–10]. In addition, a chloride requirement for taurine uptake has also been demonstrated in several tissues, including liver [6], brain [8], kidney [11,12] and in Ehrlich ascites tumor cells [7]. Two recent studies, using highly purified membrane vesicles, have established the presence of a high affinity, Na^+-coupled β-carrier system in the brush-border membrane of the human placenta [13,14]. The initial study by Miyamoto et al. [13], suggested that taurine transport was also coupled to Cl^-. Karl and Fisher [14] further examined the Cl^- dependency of the transport process controlling for changes in membrane potential. Under these conditions, they were unable to demonstrate any specific Cl^--gradient stimulation, therefore concluding that the Cl^- effect described by Miyamoto et al. was due to nonspecific changes in the membrane potential during transport and not to a direct effect of the anion itself. Our study substantiates the specific Cl^- dependence of taurine

transport in human placental brush-border membrane vesicles and further examines the Cl^- stoichiometry of Na^+/taurine symport.

Materials and Methods

Materials

[^{3}H]Taurine (17–30 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), phenylmethylsulfonyl fluoride (PMSF) and bovine serum albumin were purchased from Sigma (St. Louis, MO). All other chemicals from commercial sources were of reagent grade or the highest grade available.

Preparation of membrane vesicles. Microvillus membrane vesicles were prepared by a modified magnesium precipitation method [15]. Full-term placentas from normal vaginal deliveries were obtained within 1 h of delivery and placed on ice. All subsequent steps of fractionation were carried out at 4°C. After blood vessels and supporting stroma were excised, the remaining tissue (approx. 150 g) was washed three times with, and suspended in, 250 ml of Buffer A (300 mM mannitol, 0.1 mM PMSF, 10 mM Hepes-Tris, pH 7.4). This crude homogenate was stirred for 30 min and then filtered through 4 layers of gauze. The filtrate was centrifuged at $9150 \times g$ for 20 min and the supernatant then centrifuged at $100000 \times g$ for 30 min. The pellet from this centrifugation was resuspended in 30 ml of Buffer A with $MgCl_2$ added to a final concentration of 10 mM. The suspension was stirred for 10 min, centrifuged at $9150 \times g$ for 20 min and the resulting supernatant centrifuged at $100000 \times g$ for 30 min. This pellet was resuspended in Buffer A and again centrifuged at $100000 \times g$ for 30 min. This step was repeated three times and the final pellet was resuspended in the desired transport- or suspension buffer. Membrane vesicles were maintained on ice at 4°C and all experiments were performed within 48 h of preparation.

Transport studies. Uptake of [^{3}H]taurine was measured using a Millipore filtration technique previously described [16]. 20 μl aliquots of membrane vesicles (60–120 μg of protein) in suspension solution were equilibrated in a water bath at 25°C for 2 min. Uptake was initiated by the addition of 80 μl of incubation solution containing [^{3}H]taurine. Conditions for preloading vesicles and specific constituents of suspension and incubation buffers are outlined in the legends. All suspension, pre-incubation and incubation solutions had a pH of 7.4 and were adjusted to the same osmolarity with mannitol. Uptake was halted by the addition of 3.0 ml of ice-cold stop solution to the sample, which was then filtered through a 0.45 μm Millipore filter (HAWP, Millipore, Bedford, MA) and washed with additional stop solution. All stop solutions were adjusted to the same pH and osmolarity as the final reaction buffer. All uptakes were corrected for a blank obtained by adding stop solution to the membrane aliquot before addition of the isotope. The filter was rendered transparent with Optifluor LSC cocktail (Packard Instrument, Downers Grove, IL) and counted in a Pharmacia LKB scintillation counter.

In experiments in which the membrane potential was clamped, uptake was measured in the presence of 50 mM K^+ gluconate (or KCl) and valinomycin (5 μg/mg protein) inside and outside of the vesicles. Vesicles were preloaded by suspending in 30–40 ml of the pre-incubation buffer, collected by centrifugation at $100000 \times g$ for 30 min and then resuspended in a small volume of the pre-incubation solution. Vesicles were then allowed to equilibrate further for 2 h at room temperature.

Data points represent a minimum of three uptake determinations from at least three different membrane preparations unless otherwise indicated.

Enzyme analysis and protein determination. Activity of the microvillus membrane marker enzyme alkaline phosphatase was measured according to the method of Pekarthy et al. [17]. Na^+/K^+ adenosine triphosphatase (Na^+/K^+-ATPase) activity was determined using the method described by Scharschmidt [18] and NADPH–cytochrome-*c* reductase by the technique described by Sottacasa et al. [19]. Protein concentration was determined according to Lowry et al. [20], using bovine serum albumin as the standard.

Data analysis. Kinetic constants were derived using a weighted least-squares fit of individual data points to a rectangular hyperbola with the aid of a computer [21]. Differences of uptake rates of [^{3}H]taurine were compared using Student's *t*-test [22] and significance defined at the $P < 0.05$ level. Data are expressed as means ± standard error of the mean (S.E.). When not shown in the figures, standard error bars are contained within the symbol.

Results

The purity of the microvillus preparation was verified by enzyme marker analysis. There was a 28-fold enrichment over homogenate in the brush-border enzyme alkaline phosphatase (Table I). In contrast, the preparation was not enriched in the basolateral marker Na^+/K^+-ATPase or the microsomal enzyme NADPH–cytochrome *c*-reductase.

The presence of a Na^+-coupled transport system in the membrane preparation was confirmed by measuring the time-dependent uptake of [^{3}H]taurine in the presence and absence of an inwardly directed Na^+-gradient and by kinetic analysis. Imposition of an inwardly directed 100 mM Na^+-gradient resulted in stimulation of taurine uptake compared to uptake in the

TABLE I

Marker enzyme activities of placental homogenate and microvillus membrane preparation

Enzyme	Homogenate	Membrane	Enrichment (fold)
Alkaline phosphatase (μmol min^{-1} mg^{-1})	0.375 ± 0.045	10.725 ± 0.092	28.6
Na^+/K^+-ATPase (μmol min^{-1} mg^{-1})	0.291 ± 0.019	0.012 ± 0.006	0.06
NADPH-cytochrome-*c* reductase (nmol min^{-1} mg^{-1})	44.1 ± 2.9	19.4 ± 1.2	0.44

presence of a K^+-gradient with a transient 20-fold accumulation of substrate above the equilibrium value ('overshoot') (Fig. 1). Na^+-dependent uptake, defined as uptake in the presence of a Na^+-gradient minus uptake in the presence of a K^+-gradient, was linear from 0 to 30 s ($r = 0.99$), therefore, initial velocities were determined at 20 s.

The effect of membrane potential on taurine transport was examined by preloading the vesicles with K^+ gluconate in the presence of the K^+ ionophore valinomycin and then measuring uptake of [^{3}H]taurine with an inwardly directed 100 mM Na^+-gradient. In the presence of an outwardly directed K^+-gradient, valinomycin accelerates the efflux of K^+, inducing a transient

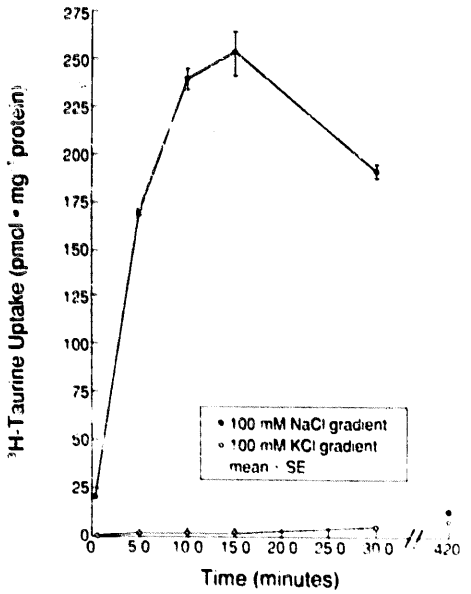

Fig. 1. Timed uptake of [^{3}H]taurine by placenta brush-border membrane vesicles. Membrane vesicles (20 μl) in a suspension buffer of 300 mM mannitol, 10 mM Hepes-Tris (pH 7.4) were incubated at 25°C for the time intervals indicated with 80 μl of incubation buffer with a final extravesicular concentration (in 100 μl) of 100 mM NaCl (•) or 100 mM KCl (○), 100 mM mannitol, 10 mM Hepes-Tris (pH 7.4) and 1 μM [^{3}H]taurine. Uptakes represent the means ± S.E. of triplicate determinations from three separate membrane preparations.

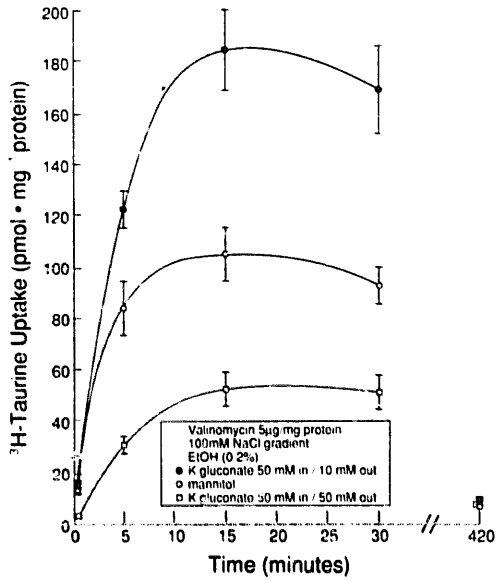

Fig. 2. Effect of membrane potential on the uptake of [^{3}H]taurine. Membrane vesicles were suspended and pre-incubated for 2 h at room temperature in 350 mM mannitol, 10 mM Hepes-Tris (pH 7.4) (○) or 50 mM K^+ gluconate, 250 mM mannitol, 10 mM Hepes-Tris (pH 7.4) (• and □) with valinomycin in ethanol added to a final concentration of 5 μg/mg protein. 20-μl aliquots of membrane vesicles were then incubated at 25°C for the time intervals indicated with 80 μl of incubation solution with a final extravesicular concentration (in 100 μl) of 1 μM [^{3}H]taurine, 100 mM NaCl, 10 mM Hepes-Tris (pH 7.4) and 10 mM K^+ gluconate, 130 mM mannitol (•), 50 mM K^+ gluconate, 50 mM mannitol (□), or 150 mM mannitol (○). Uptakes represent the means ± S.E. of triplicate determinations from three separate membrane preparations.

negative intravesicular potential. These data are compared with uptake measured under "voltage-clamped' conditions in which intra- and extravesicular concentrations of K^+ with valinomycin were equal and with control uptakes measured in the presence of valinomycin but without K^+ in the buffer solutions. As shown in Fig. 2, a negative intravesicular potential significantly enhanced the initial rate and peak uptake of taurine compared with uptake under control conditions. In addition, clamping the membrane potential at zero resulted in a significant decrease in the initial velocity of taurine uptake, as compared to control values. These data suggest that transport is sensitive to the electrical potential across the membrane.

The kinetics of Na^+-dependent taurine uptake were performed by measuring initial uptake velocity (20 s) over a range of concentrations from 0.5 to 100.0 μM. For these studies, the potential across the membrane was clamped at zero to minimize the effect of transmembrane potential on carrier-mediated uptake. Na^+-dependent taurine transport was saturable (Fig. 3) and a single carrier system was described (inset, Fig. 3) with a calculated apparent K_m of 4.00 ± 0.22 μM and a V_{max} of 11.72 ± 0.36 pmol mg^{-1} protein 20 s^{-1}.

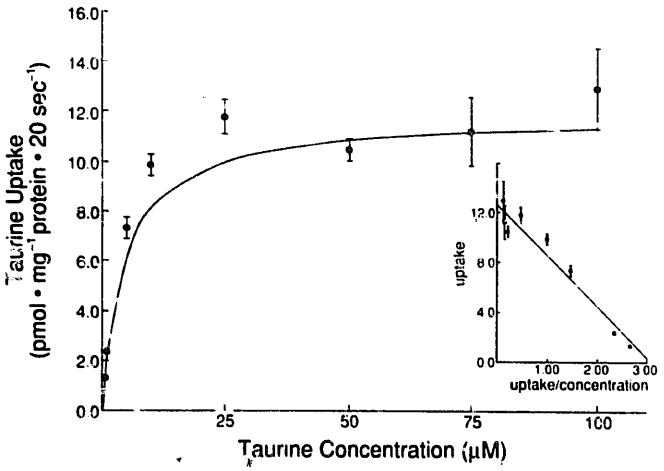

Fig. 3. Kinetics of Na^+-dependent taurine uptake by placental brush-border membrane vesicles. Membrane vesicles were suspended and pre-incubated at room temperature for 2 h in 250 mM mannitol, 50 mM KCl, 10 mM Hepes-Tris (pH 7.4) and valinomycin (5 μg/mg protein). 20-μl aliquots of membrane vesicles were then incubated at 25°C for 20 s (initial velocity) with 80 μl of incubation buffer with a final extravesicular concentration (in 100 μl) of 50 mM KCl, 50 mM mannitol, 10 mM Hepes-Tris (pH 7.4) and either 100 mM NaCl or an additional 100 mM KCl and concentrations of taurine ranging from 0.5 to 100 μM with [^{3}H]taurine as a tracer. Na^+-dependent uptake was defined as uptake in the presence of a Na^+-gradient minus uptake in the presence of a K^+-gradient. Weighted least-squares fit of individual data points to a rectangular hyperbola was performed with the aid of a computer. The Eadie-Hofstee transformation of the data is shown in the inset. Data points represent the means ± S.E. of multiple determinations at each concentration from several membrane preparations.

The ion requirements of the carrier system were then examined in more detail. In agreement with previous studies, the transport process had an absolute requirement for Na^+, with substitution of other cations (lithium, choline, K^+) resulting in minimal uptake under voltage-clamped conditions (Table II). The Na^+-gradient, not just Na^+ per se, was required to energize uphill transport of taurine, since no overshoot could be demonstrated under NaCl-equilibrated conditions, although uptake was significantly enhanced over that obtained in the absence of Na^+ (Fig. 4).

In addition to Na^+, there was also a specific anion requirement for optimum transport function. With the exception of Br^-, substitution of Cl^- with other anions resulted in substantially decreased uptake and loss of the overshoot (Table III). Uptake in the presence of Br^- was qualitatively similar, but there was still a statistically significant decrease in uptake velocities, as compared to those in the presence of a Cl^--gradient. This stimulation was not specifically dictated by anion permeability or electrical effects since the membrane potential was again clamped for these experiments. Because these results contradicted those previously reported by Karl and Fisher [14], we repeated these experiments using KSCN rather than K^+ gluconate to

TABLE II

Effect of cation substitution on the uptake of [^{3}H]taurine (1 μM)

Membrane vesicles were suspended and pre-incubated at room temperature for 2 h in a buffer of 50 mM K^+ gluconate, 250 mM mannitol, 10 mM Hepes-Tris (pH 7.4) and valinomycin (5 μg/mg protein). 20-μl aliquots were then incubated at 25°C for the designated time intervals in 80 μl of incubation buffer with a final extravesicular concentration (in 100 μl) of 100 mM Cl^-, 100 mM of the cation indicated, 50 mM K^+ gluconate, 10 mM Hepes-Tris (pH 7.4) and 1 μM [^{3}H]taurine. Data represent the means ± S.E. of triplicate determinations from two separate membrane preparations.

Cation	Uptake (pmol mg^{-1} protein)		
	20 s	15 min	300 min
NaCl	6.57 ± 0.14	95.00 ± 4.50	13.90 ± 0.44
KCl	0.14 ± 0.04 [a]	2.82 ± 0.14	9.21 ± 0.45
LiCl	0.22 ± 0.03 [a]	2.19 ± 0.07	9.59 ± 0.30
Choline chloride	0.11 ± 0.02 [a]	1.86 ± 0.18	8.35 ± 0.17

[a] $P < 0.001$ compared to NaCl.

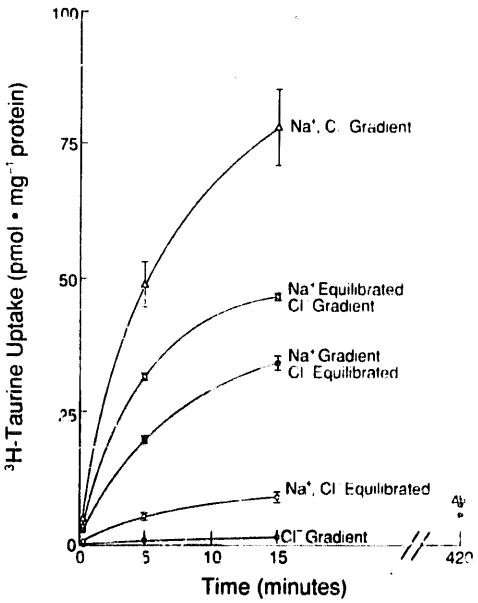

Fig. 4. Effect of Na^+ and Cl^- on timed uptake of [^{3}H]taurine. Membrane vesicles were suspended in buffers containing 50 mM K^+ gluconate, 10 mM Hepes-Tris (pH 7.4), valinomycin (5 μg/mg protein) and the following: (1), 250 mM mannitol; (2), 100 mM Na^+ gluconate, 50 mM mannitol; (3), 100 mM choline chloride, 50 mM mannitol and (4), 100 mM NaCl, 50 mM mannitol. Valinomycin was added (5 μg/mg protein) and the membranes were pre-incubated at room temperature for 2 h. 20-μl-aliquots of membrane were then incubated at 25°C for the time intervals indicated in 80 μl of incubation solutions containing 50 mM K^+ gluconate, 10 mM Hepes-Tris (pH 7.4) and the following: (△), 125 mM NaCl (suspension 1); (□), 100 mM NaCl, 25 mM choline chloride (suspension 2); (■), 100 mM NaCl, 25 mM Na^+ gluconate (suspension 3); (○), 100 mM NaCl, 50 mM mannitol (suspension 4) and (●), 125 mM choline chloride (suspension 1) with 1.25 μM [^{3}H]taurine added (final concentration 1 μM). The final concentration of Na^+ and/or Cl^- (in 100 μl) was 100 mM. Data points represent the means ± S.E. of triplicate determinations from two separate membrane preparations.

TABLE III

Effect of anion substitution on the uptake of [^{3}H]taurine (1 μM)

Membrane vesicles were pre-incubated and uptake was measured as in Table II. Incubation buffer (80 μl) was added with a final extravesicular concentration (in 100 μl) of 100 mM Na^+, 100 mM of the anion indicated, 50 mM K^+ gluconate, 10 mM Hepes-Tris (pH 7.4) and 1 μM [^{3}H]taurine. Data represent the means ± S.E. of triplicate determinations from two separate membrane preparations.

Anion	Uptake (pmol mg^{-1} protein)		
	20 s	15 min	300 min
NaCl	6.57 ± 0.14	95.00 ± 4.50	13.90 ± 0.44
NaBr	4.67 ± 0.31 [a]	77.31 ± 6.01	13.99 ± 0.40
NaSCN	2.24 ± 0.33 [a]	19.23 ± 1.47	12.84 ± 0.24
$NaNO_3$	0.65 ± 0.14 [a]	14.70 ± 0.19	16.45 ± 0.51
Sodium cyclamate	0.24 ± 0.02 [a]	4.76 ± 0.46	11.51 ± 0.84
Sodium gluconate	0.30 ± 0.20 [a]	2.28 ± 0.22	8.50 ± 0.13

[a] $P < 0.001$ compared to NaCl.

clamp the membrane potential, pre-incubating the vesicles at 37°C for 30 min and measuring uptake in the presence of a 50 mM rather than 100 mM ion gradient as described in their study. Uptake rates were lower using this experimental design, however, a significant difference in uptake in the presence of a NaCl gradient, as compared to a NaSCN gradient, was again demonstrated (Table IV).

Uptake was then measured under Cl^--equilibrated and Cl^--gradient conditions, in the presence and absence of Na^+, with the membrane potential clamped. As shown in Fig. 4, uphill transport of taurine could be

TABLE IV

Comparison of the effects of a Cl⁻ vs. a SCN⁻ gradient on uptake of [^{3}H]taurine (1 μM) in the presence and absence of Na^+

Membrane vesicles were suspended in buffer containing 50 mM KSCN, 200 mM mannitol, 10 mM Hepes-Tris (pH 7.4) and valinomycin (5 μg/mg protein) and pre-incubated at 37°C for 30 min. 20-μl aliquots of vesicles were then incubated at 25°C for 2 min in incubation solution (80 μl) with a final concentration (in 100 μl) of 50 mM KSCN + 50 mM NaCl, 50 mM KSCN + 50 mM NaSCN, 50 mM KSCN, or 50 mM KCl and 10 mM Hepes-Tris (pH 7.4) with the osmolarity adjusted to 310 mOsm with mannitol. Na^+-dependent uptake was obtained by subtracting the mean of uptake in the absence of Na^+ from individual uptakes in the presence of Na^+ and the corresponding anion. Data represent the means ± S.E. of 15 determinations from five different membrane preparations for uptake in the presence of Na^+ and 12 determinations from four separate preparations for uptake in the absence of Na^+.

Ions (50 mM) inside/outside	Total	
	Uptake (pmol mg^{-1} protein 2 min^{-1})	Na^+-dependent
KSCN/NaCl + KSCN	2.17 ± 0.12	1.47 ± 0.12 [a]
KSCN/NaSCN + KSCN	1.15 ± 0.05	0.57 ± 0.05 [a]
KSCN/KSCN	0.58 ± 0.03	
KSCN/KCl	0.70 ± 0.03	

[a] $P < 0.001$.

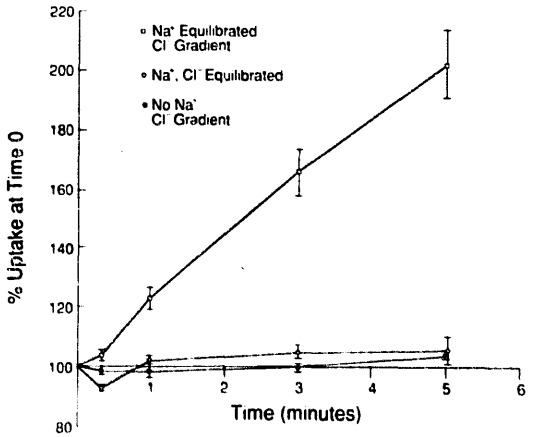

Fig. 5. The effect of a Cl^--gradient on the uptake of [^{3}H]taurine. Membrane vesicles were suspended and pre-incubated at room temperature for 2 h in suspension solutions containing 1 μM [^{3}H]taurine, 10 mM Hepes-Tris (pH 7.4), valinomycin (5 μg/mg protein) and the following: (1), 100 mM Na^+ gluconate, 50 mM K^+ gluconate; (2), 100 mM NaCl, 50 mM K^+ gluconate or (3), 150 mM K^+ gluconate. 20-μl aliquots of membrane were then incubated at 25°C for the time intervals indicated in 80 μl of incubation buffers with a final extravesicular concentration (in 100 μl) of 1 μM [^{3}H]taurine, 50 mM K^+ gluconate, 10 mM Hepes-Tris (pH 7.4) and either 100 mM NaCl (suspension 1 (□) or suspension 2 (○)) or 100 mM choline chloride (suspension 3 (●)). Uptakes represent the means ± S.E. of triplicate determinations from three separate membrane preparations.

demonstrated in the presence of either a Na^+-gradient (under Cl^--equilibrated conditions) or a Cl^--gradient (under Na^+-equilibrated conditions), indicating coupling of taurine transport with both Na^+- and Cl^--influxes. Uptake in the absence of Na^+, even with a Cl^--gradient, was again negligible.

To further substantiate this direct effect of the Cl^--gradient on the transport process, uptake of [^{3}H]taurine (1 μM) was measured in vesicles preloaded with [^{3}H]taurine (1 μM) under Cl^--gradient and Cl^--equilibrated conditions. The membrane potential was clamped and the vesicles were Na^+-equilibrated (Fig. 5). In the presence of an inwardly directed Cl^--gradient, [^{3}H]taurine accumulated against its concentration gradient, while uptake under Cl^--equilibrated conditions (or under Cl^--gradient conditions in the absence of Na^+) was not enhanced beyond baseline conditions (measured at time 0).

Previous studies, in both placenta [14] and other epithelial tissues [7,11], have demonstrated an approximate 2:1 Na^+ : taurine stoichiometry for brush-border membrane transport. Because our data suggest a stimulatory role for Cl^- as well, we examined the stoichiometry of the interaction of Cl^- with the Na^+ : taurine co-transporter. In voltage-clamped vesicles, initial rates of taurine uptake (1 μM) were measured at varying concentrations of external Cl^- with a constant extravesicular concentration of Na^+ (250 mM) and the results are shown in Fig. 6. Data points con-

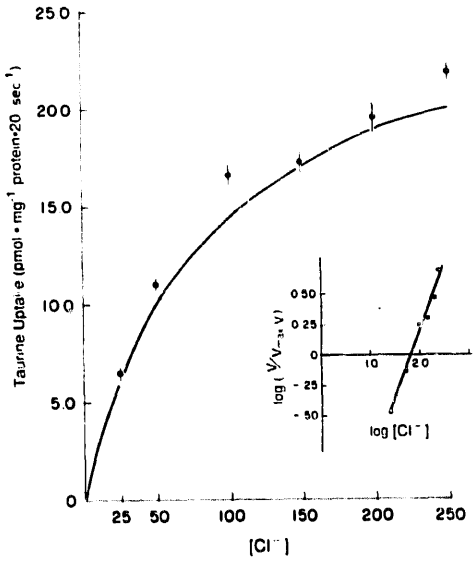

Fig. 6. The effect of varying concentrations of external Cl^- on the uptake of [^{3}H]taurine. Membrane vesicles were suspended and preincubated at room temperature in a solution containing 625 mM mannitol, 50 mM K^+ gluconate, 10 mM Hepes-Tris (pH 7.4) and valinomycin (5 μg/mg protein). 20-μl aliquots of membrane were then incubated at 25°C for 20 s (initial velocity) in 80 μl of buffer containing 50 mM K^+ gluconate, 10 mM Hepes-Tris (pH 7.4), 1 μM [^{3}H]taurine (final concentration) and varying concentrations of Na^+ gluconate and NaCl to give a final concentration of 250 mM Na^+ and from 0 to 250 mM Cl^- (in 100 μl). Uptakes represent the means ± S.E. of multiple determinations from several separate membrane preparations. Hill plot analysis of the data points is shown in the inset.

form to a rectangular hyperbola and the line derived by Hill plot analysis of the data (shown in the inset) was defined by the equation $y = 1.09x - 1.99$. The slope of the line (1.09) indicates an approximate Cl^-/taurine stoichiometry of 1 : 1.

Discussion

Taurine appears to be essential for normal fetal growth and development [1–4,23,24]. The capacity for endogenous taurine biosynthesis in the fetus is limited, therefore, the placental transfer of this amino acid should play an important role in maintaining fetal taurine homeostasis. Taurine is the most abundant free amino acid in the human placenta with a concentration of approx. 3.5 μmol/g wet weight or 10.43 μmol/ml. This represents a significant concentration gradient when compared to taurine levels in maternal (0.060 μmol/ml) and fetal (0.135 μmol/ml) plasma [25], suggesting active mediation of transport across placental membrane domains.

Transport of taurine across the apical membrane in several tissues and cell lines has been well described [6–14] and involves a relatively high-affinity, Na^+-coupled, carrier-mediated process specific for β-amino acids. Cl^--dependence of this β-carrier has also been suggested in several of these studies and is particularly well-defined in the renal brush-border membrane [11,12,26]. Recently, two studies have described a similar high-affinity, Na^+-dependent, β-amino acid transport system in the human placenta using purified brush-border membrane vesicles [13,14]. Miyamoto et al. [13] suggested that taurine transport was also coupled to an inwardly directed Cl^--gradient. This Cl^--dependence was inferred from enhanced initial rates of uptake in the presence of an inwardly directed Cl^--gradient compared to uptake either under Cl^--equilibrated conditions or in the absence of Cl^- (inwardly directed NaF-gradient). In the study by Karl and Fisher [14], the affinity of the carrier, derived from kinetic analysis in membrane vesicles (apparent K_m 6.2 ± 0.7 μM), was similar to that reported by Miyamoto (6.5 ± 0.4 μM), as were the dependence on Na^+ and selective inhibition by other β-amino acids. In addition, a stoichiometry of 2 : 1 was defined for Na^+/taurine cotransport. However, when transport experiments were performed with the membrane potential clamped at zero, no stimulation of uptake by a Cl^--gradient, either in the presence or absence of Na^+, could be demonstrated. From these data, the authors concluded that Cl^- was not actively involved in transport, but served only a passive role dictated by anion permeability and membrane potential.

Our study again supports the presence of a specific transport system for taurine in the placental brush-border membrane and the kinetic parameters defined by our data (apparent K_m 4.00 ± 0.22 μM, V_{max} 11.72 ± 0.35 pmol mg^{-1} protein 20 s^{-1}) are comparable to those published by Karl and Fisher (K_m 6.5 ± 0.4 μM, V_{max} 24.5 ± 0.6 pmol mg^{-1} protein 30 s^{-1}). Taurine uptake was dependent on Na^+ with substitution of other cations, such as choline, K^+ and Li^+, resulting in minimal transport.

In contrast to Karl and Fisher's study, we have demonstrated that a Cl^--gradient can also stimulate transport of taurine across the placental brush-border membrane and that the concentration gradient, not just the presence of Cl^- alone, is required for this stimulation. However, even an inwardly directed Cl^--gradient could not energize uptake in the absence of Na^+, suggesting a functional interaction among Na^+, Cl^- and substrate at the carrier site and subsequent co-transport of all three across the membrane. These effects could not be attributed to changes in membrane potential, since the voltage across the membrane was clamped at zero in these experiments. When other anions were substituted for Cl^-, again under clamped conditions, uphill transport could be demonstrated, although to a lesser degree, for bromide but not for thiocyanate, gluconate, cyclamate or nitrate. Analysis of taurine uptake in the presence of varying concentra-

tions of external Cl^- (and a fixed concentration of Na^+) suggested a stoichiometric interaction of 1:1 for Cl^-/taurine transport. Since taurine exists as a zwitterion at physiologic pH, a stoichiometry of 2:1:1 for Na^+/Cl^-/taurine symport would be in agreement with the electrogenic nature of the transport process.

These results differ from those reported by Karl and Fisher who did not demonstrate any specific Cl^- effect when a zero membrane potential was imposed. This may be related to several factors. Uptake rates are significantly lower when the membrane potential is clamped. In addition, experimental conditions, such as pre-incubation at 37°C and measuring uptake in the presence of a 50 mM rather than a 100 mM gradient, may further lower these rates. Under these conditions, small but significant differences in uptake may be more difficult to demonstrate. Although the enzyme marker profile of our membrane vesicles is similar to that reported by Karl and Fisher, there appears to be significantly less basolateral membrane contamination in our preparation as reflected by the lack of enrichment in Na^+/K^+-ATPase compared to an approx. 3.8-fold enrichment in theirs. This suggested enhanced purity of our preparation may be sufficient to allow demonstration of these significant differences in transport, even when uptake rates are decreased by imposed experimental conditions.

In summary, a high-affinity, electrogenic, Na^+-dependent transport system for taurine is present in the brush-border membrane of the human placenta. Our data also support a selective anion requirement of this Na^+/taurine symport with maximal uptake in the presence of external Cl^-. Kinetic analysis of the activation of taurine uptake by Cl^- is consistent with a transport stoichiometry of 2:1:1 (Na^+/Cl^-/taurine). These functional properties are similar to those described for taurine transport in the apical membrane of other epithelial tissues and suggest structural similarities in these transport proteins.

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