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Factors affecting the transport of β-amino acids in rat renal brush-border membrane vesicles. The role of external chloride

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The effect of a variety of ions and other solutes on the accumulation of the β-amino acid, taurine, was examined in rat renal brush-border membrane vesicles. Initial taurine uptake (15 and 30 s) is sodium-dependent with a typical overshoot. This Na^+ effect was confirmed by exchange diffusion and gramicidin inhibition of taurine uptake. External K^+ or Li^+ do not increase taurine accumulation more than Na^+-free mannitol, except that the combination of external K^+ and Na^+ in the presence of nigericin enhances uptake. Of all anions tested, including more permeant (SCN^- and NO_3^-) or less permeant (SO_4^{2-}), chloride supported taurine accumulation to a significantly greater degree. Preloading vesicles with choline chloride reduced taurine uptake, suggesting that external Cl^- stimulates uptake. Since this choline effect could be related to volume change, due to the slow diffusion of choline into vesicles, brush-border membrane vesicles were pre-incubated with LiCl, $LiNO_3$ and $LiSO_4$. Internal LiCl, regardless of the final Na^+ anion mixture, reduced initial rate (15 and 60 s) and peak (360 s) taurine uptake. Internal $LiNO_3$ or $LiSO_4$ with external NaCl resulted in similar or higher values of uptake at 15, 60 and 360 s, indicating a role for external Cl^- in taurine uptake in addition to Na^+ effect. Although uptake by vesicles is greatest at pH 8.0 and inhibited at acidic pH values (pH less than 7.0), an externally directed H^+ gradient does not influence uptake. Similarly, amiloride, an inhibitor of the Na^+/H^+ antiporter, had no influence on taurine accumulation over a wide variety of concentrations or at low Na^+ concentrations. Taurine uptake is blocked only by other β-amino acids and in a competitive fashion. D-Glucose and *p*-aminohippurate at high concentrations ($> 10^{-3}$ M) reduce taurine uptake, possibly by competing for sodium ions, although gramicidin added in the presence of D-glucose inhibits taurine uptake even further. These studies more clearly define the nature of the renal β-amino acid transport system in brush-border vesicles and indicate a role for external Cl^- in this uptake system.

Introduction

The β-amino acid transport system in kidney is utilized to transfer the β-amino acids β-alanine, β-aminoisobutyrate and taurine, as well as the γ-amino acid γ-aminobutyrate across the tubular epithelium [1]. These β-amino acids inhibit the uptake of one another in a competitive fashion in slices and tubules [2–4] but do not interact with α-amino acids or other organic solutes. A sodium (Na^+) electrochemical gradient-dependent β-amino acid transport system has been reported in

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renal brush-border membrane vesicles having a specificity for β-amino acids in mice, rabbits and rats [5–8]. The accumulation of β-alanine and taurine is rather slow compared to that of D-glucose, since the peak of the overshoot appears at 4 to 7 min [5–7]. Other characteristics of β-amino acid transport by brush-border membrane vesicles have not been reported. In this study, we have examined the characteristics of taurine accumulation by brush-border membrane vesicles.

Methods

Animals. Sprague-Dawley rats (King Labs, Fitchburg WI) aged 58 to 60 days and weighing 150–175 g were used in all studies.

Membrane vesicle preparation. Renal cortex brush-border membrane vesicles were isolated by a series of differential centrifugations using a modification of the method of Booth and Kenny [9]. Rats were placed under anhydrous ether anesthesia and exsanguinated; their kidneys were removed, decapsulated and placed in cold (4°C) saline) Renal cortex tissue was cut away. Samples of between 1.5 and 6 g wet weight were homogenized in 20 volume of 0.05 M D-mannitol, 2 mM Tris-HCl (pH 7.4) and 10 mM Hepes (Tris-Hepes-mannitol) for 5 min with a Sorvall omnimixer (setting 10) in an ice bath. Calcium chloride (final concentration 10 mM) was added to aggregate the intracellular and basolateral membranes and stirred on ice for 15 min. The details of membrane vesicle preparation are given elsewhere [7,10]. Membrane vesicles were used for uptake studies on the day of preparation.

Enzyme and protein determinations. Membrane purity was routinely assessed from the enrichment of γ-glutamyltransferase and 5′-nucleotidase, markers for brush-border membranes [11,12]. Other enzymes examined were ouabain-inhibitable $(Na^+ + K^+)$-ATPase [13] as a marker of basolateral membranes; lactate dehydrogenase [14] as a marker of microsomal membranes; succinyl cytochrome-*c* reductase [15] as a mitochondrial marker; acid (pH 4.8) phosphatase [16] and *N*-acetyl-β-D-glucosaminidase [17] to indicate lysosomes, and DNA [18] to indicate nuclei. Protein was determined by the method of Lowry et al. [19] after precipitation in 6% trichloroacetic acid.

Amino acid uptake studies. Uptake of radioactive [^{3}H]taurine and β-[^{14}C]alanine was assayed by a Millipore filtration technique [20]. In general, 200 μg of membrane suspension was preincubated at 25°C for 30 to 45 min. Incubation was initiated by the addition of medium containing known amounts of unlabelled and radiolabelled taurine or β-alanine; usually 0.5 μCi was added. All incubation media contained 300 mM mannitol, 1 mM $MgSO_4$, 10 mM Hepes/Tris (pH 7.4) and the other salts noted. After the desired time interval, a 50-μl aliquot was placed on a prewetted 0.45-μm Millipore filter (HAWP). The filtered sample was washed twice with 3.0 ml of iced wash solution; this entire process took 12 s. The iced 'stop solution' contained 300 mM mannitol, 1 mM $MgSO_4$ and Hepes/Tris (pH 7.4). Filters were dried overnight in scintillation flasks and then dissolved in Aquasol and counted for radioactivity in a liquid scintillation counter. The values for the nonspecific retention of radioactivity were subtracted from values obtained after incubation with membranes. The number of cpm obtained using [^{3}H]taurine seldom exceeded 20 cpm and, since more than 10^3 cpm were retained on the filter in most cases, this nonspecific retention was about 2% of the specific amount found.

Analytical. Data comparisons were made with Student's *t*-test, linear regression analysis and analysis of variance using a desk-top computer with established programs (Texas Instruments Users Guide). Analysis of kinetics for transport functions was performed using WISAR (Madison, WI) modification of the method of Neal [21].

Materials. [^{3}H]Taurine (spec. act. 23.1 Ci/mM) and β-alanine were purchased from New England Nuclear (Boston, MA). Radiochemical purity was confirmed by one-dimensional TLC. All chemicals used to prepare media were reagent grade.

Results

Analysis of brush-border membrane preparations from 58–60-day-old rats showed γ-glutamyltransferase and 5′-nucleotidase (markers of the brush-border surface) were 8.2- to 11.4-fold ($n = 30$) and 5.9-fold ($n = 30$) enriched relative to the starting homogenate. No enrichment relative to the starting homogenate was found for other cell

membrane markers. The initial rate (15 and 60 s) of taurine uptake (10 μM), in the presence of a Na^+ gradient, was proportional to the concentration of membrane protein over the range of 5 to 13 mg/ml.

The uptake of taurine (50 μM) in the presence of an external Na^+ gradient (100 mM) was up to 30-times higher than in the presence of a 100 mM LiCl gradient (Fig. 1). An overshoot was not observed when the vesicles was preloaded with 100 mM NaCl in the absence of any external cation (289 mosM mannitol, 10 mM Tris-Hepes, 1 mM Mg_2SO_4) or in the presence of a 100 mM KCl gradient. The uptake of taurine in the presence of external LiCl or KCl was not different, but was slightly higher than in the presence of mannitol ($p < 0.01$) (Table I). Although LiCl or KCl support this slightly higher level of taurine uptake, the accumulation certainly appears to be Na^+-dependent.

The uptake of taurine by brush-border membrane vesicles was measured in the presence of external NaCl (100 mM) at various time intervals (15, 30, 45, 60 and 90 s) over the time-course of uptake at several concentrations (10, 25 and 50 μM) (Fig. 1). When uptake was compared in terms of concentration dependency at 15 s vs. 60 s, several findings became apparent. Although a large range of taurine concentrations was examined (10 μM to 5 mM), only one active uptake component was apparent, as we previously reported [7]. The K_m of uptake (Na^+-dependent component) was the same at 15 s and 60 s (38 and 42 μM, respectively) using Eadie-Hoffstee analysis. The uptake rate is higher at 15 s than at 60 s when expressed in terms of a 60-s uptake rate, but the range of uptake values and the standard deviation of uptake are greater at 15 s so that these difference achieve minimal significance. For example, at 25 μM taurine, the uptake at 15 s is 44.84 ± 7.6 (S.D.) pmol/mg proten per 60 s when expressed over 60 s vs. 29.65 ± 2.21 pmol/mg protein per 60 s when measured over 60 s, $p < 0.05$. Hence, for most experiments, we have chosen a 60-s sampling time.

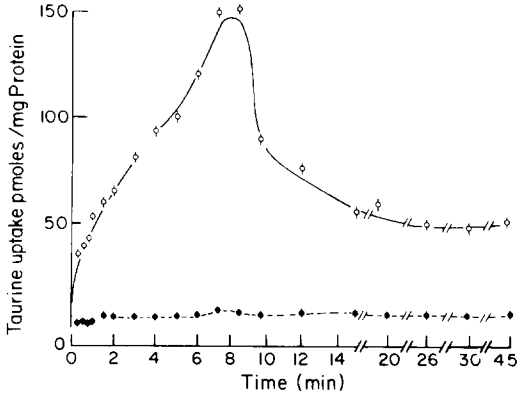

Fig. 1. Uptake over time of [^{3}H]taurine and unlabelled taurine (50 μM, final concentration) in brush-border membrane vesicles in the presence (○) and absence (●) of a 100 mM external NaCl gradient. 100 mM LiCl (●) was used to replace NaCl in the incubation. Each point represents the mean ± S.E. of 12–20 determinations.

TABLE I

TAURINE UPTAKE (10 μM) IN THE PRESENCE OF VARIOUS SALTS IN THE EXTERNAL MEDIUM

Values represent means ± S.E. (pmol/mg protein), with $n = 12$ in each case. Vesicles were prepared in Tris-Hepes, pH 7.4 (10 mM), $MgSO_4$ (1 mM) and mannitol (2989 mosM). Incubation took place in the presence of 100 mM of the salt. n.s., not significant.

	60 s	360 s	p (60 vs. 360 s)
NaCl	27.40 ± 1.05	54.75 ± 1.99	0.001
KCl	2.01 ± 0.18 [a,b]	4.50 ± 0.22 [a,b]	0.001
LiCl	1.77 ± 0.22 [a,b]	4.19 ± 0.18 [a,b]	0.001
Mannitol	1.26 ± 0.03 [b,c]	2.25 ± 0.17 [b,c]	n.s.

[a] KCl vs. LiCl: not significant.
[b] Mannitol vs. NaCl, KCl vs. NaCl and LiCl vs. NaCl: $p < 0.0002$.
[c] KCl vs. mannitol, LiCl vs. mannitol: $p < 0.01$.

Additional evidence for transport of taurine into vesicles came from the use of the technique of exchange diffusion. Brush-border membrane vesicles were preloaded with unlabelled 2 mM taurine in the 300 mosM Tris-Hepes-mannitol medium or in the absence of the amino acid (control). At time zero, traces of [^{3}H]taurine were added, and the 60-s rate of taurine was measured. Compared to the uptake in control of 74.18 ± 1.33 pmol/mg protein per min ($n = 24$), marked stimulation in the initial rate of taurine uptake (406 ± 5%, $n = 24$), was evident when the brush-border membrane vesicles were preloaded with taurine. Since accelerated exchange diffusion is present, it

is likely that the accumulation of taurine by brush-border membrane vesicles represents transport into vesicles rather than occupation of binding sites. The Na^+ ionophore, gramicidin, blocks the uptake of taurine by brush-border-membrane vesicles during the initial uptake phase, but not after equilibrium has been achieved (Table II). Thus, dissipation of the sodium gradient, in the initial phase of taurine accumulation, appears to significantly reduce the uptake of taurine.

The non-electrogenic K^+-H^+ exchange ionophore, nigericin, results in an augmentation of taurine accumulation under certain conditions. With NaCl alone (control condition), the uptake of taurine was 36.41 ± 0.73 pmol/mg protein per 60 s ($n = 12$). Nigericin (100 μg/ml) added to this mixture results in an uptake of 36.39 ± 0.59 pmol/mg protein per 60 s, not different from control. Nigericin (100 μg/ml) plus external 23 mM KCl (with appropriate reduction in mannitol concentration) results in an uptake of 45.5 ± 0.91 pmol/mg protein per 60 s, $P < 0.001$ vs. control. Preincubation in the presence of KCl (23 mM), with incubation using nigericin and external NaCl (100 mM), results in a lower uptake value of 31.6 ± 0.57 pmol/mg protein per 60 s, $p < 0.01$. Incubation with both internal (preincubation) and external (incubation) KCl (23 mM) in the incubation mixture gives an uptake value of 42.16 ± 0.63 pmol/mg protein per 60 s, significantly higher than the value in control ($P < 0.01$). Thus, external KCl in addition to NaCl enhances uptake in the presence of nigericin.

The influence of various anions on taurine uptake is shown in Fig. 2. The membrane potential was varied using different sodium salts. Uptake in the presence of the relatively impermeant anion, SO_4^{2-}, was markedly reduced as compared to the uptake in the presence of a NaCl gradient. Thus, uptake into a membrane whose inside is less negative is reduced [22]. If the Na^+ gradient uptake of solutes were electrogenic, the uptake of taurine should be lower with a less negative inside of the vesicle. However, using NaSCN or $NaNO_3$, which are more permeant, one would anticipate a higher intravesicular negativity [23]. Yet, despite this higher intravesicular negativity, the accumulation of taurine is again reduced (Figs. 2A and 2C). Other sodium anions also were examined (sodium tartarate, sodium acetate and sodium pyrophosphate) none of which supported taurine uptake to a level higher than that found using sodium sulfate (data not shown). Indeed, the accumulation of taurine is clearly greatest in the presence of sodium chloride.

In order to determine whether taurine transport is chloride-dependent the effect of intravesicular chloride on taurine uptake was examined. Vesicles were preloaded with choline chloride (100 mM) and 100 mosM Tris-Hepes-mannitol for 30 min

TABLE II

INFLUENCE OF GRAMICIDIN ON TAURINE ACCUMULATION

Brush-border membrane vesicles were incubated in 100 mM NaCl, 97 mM mannitol, 2 mM Tris-Hepes (pH 7.4), 1 mM $MgSO_4$ and 10 μM taurine for the times shown. Taurine uptake values represent means ± S.E. of six experiments ($n = 6$) performed in triplicate.

Time	Condition	Taurine uptake (pmol/mg protein)
60 s	Control	32.97 ± 1.64
60 s	+20.3 μg gramicidin per mg protein	23.30 ± 0.55 [a]
45 min	Control	11.99 ± 0.44
45 min	+20.3 μg gramicidin per mg protein	13.07 ± 1.68

[a] $p < 0.01$.

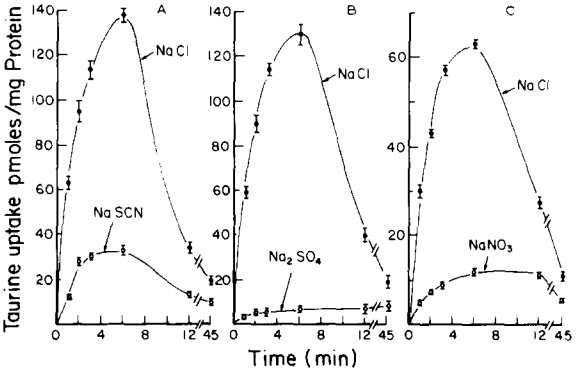

Fig. 2. Uptake over time of taurine (10 μM) in the presence of various sodium salts. Uptake by brush-border membrane vesicles is examined in the presence of: (A) NaSCN, (B) Na_2SO_4, and (C) $NaNO_3$. Each point represents the mean ± S.E. of 12 determinations.

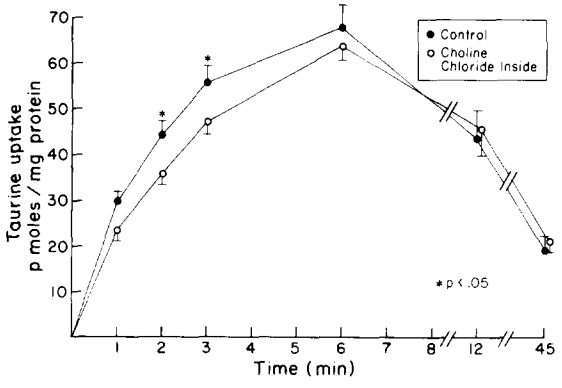

Fig. 3. Uptake over time of taurine (10 μM) in the presence (○) or absence (●) of an internal choline chloride prior to incubation in an external 100 mM NaCl gradient. Each point represents the mean ± S.E. of 12 determinations.

and then incubated in a medium containing external 100 mM NaCl. The time-dependent uptake of 10 μM taurine was reduced as compared to vesicles preincubated in 300 mosM Tris-Hepes-mannitol (Fig. 3). A reduction of approx. 20% was evident between 60 and 360 s. When vesicles were preloaded with choline sulfate or thiocyanate, no change in taurine uptake was found upon subsequent incubation in a 100 mM NaCl external medium, the values being within 2% of those obtained using NaCl alone.

Since choline is a slowly permeant cation and might influence uptake due to volume effects, we employed other salts (Na^+ and Li^+) to define the relative roles of internal and external Cl^- on taurine accumulation (Fig. 4). Chloride was preloaded into the vesicles using NaCl or LiCl in the 30-min preincubation phase. Compared to control conditions (mannitol in/NaCl out), taurine uptake after the addition of a variety of external salts (NaCl, $NaNO_3$, NaSCN, LiCl and $LiSO_4$) during the incubation step is significantly reduced at 15, 60 and 360 s. Uptake is minimal when the preincubation/incubation combination consists of Li^+/Li^+. By contrast, after preincubation in $LiNO_3$ or $LiSO_4$, addition of external NaCl results in taurine accumulation similar to ($LiNO_3$) or higher than ($LiSO_4$) the mannitol/NaCl control at the same time intervals. These results are consistent with the notion that external Cl^- in the presence of Na^+ results in maximal taurine uptake.

The pH dependency of taurine accumulation is shown in Table III. Preincubation of vesicles at pH 5.5 and then incubation at pH 7.4 does not

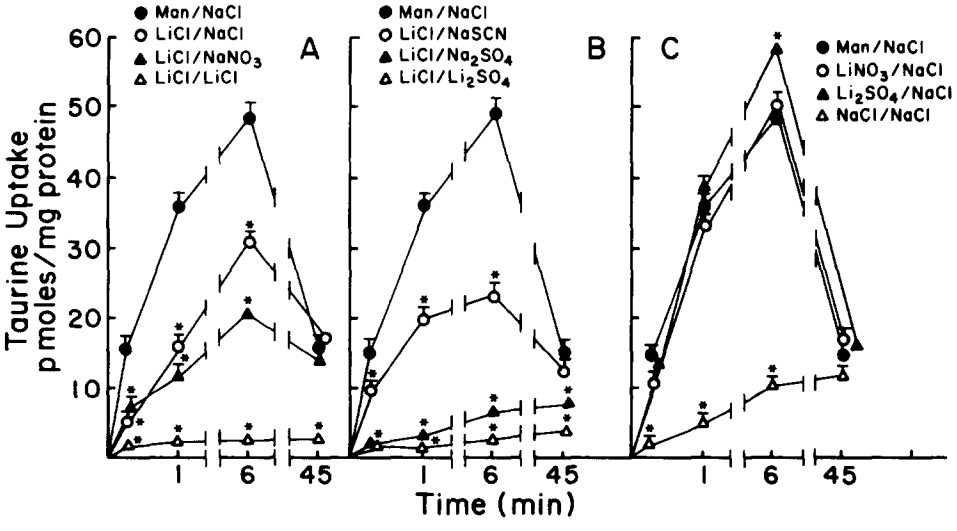

Fig. 4. The effect of various Li^+ and Na^+ salts on taurine uptake over time. Shown to the left of the symbol legend is the preincubation salt, with the incubation salt to the right. Brush-border membrane vesicles were preincubated in a medium containing 100 mM Na or Li salt, 89 mM mannitol, 10 mM Tris-Hepes (pH 7.4), 1 mM $MgSO_4$ for 30 min. The incubation mixture included 100 mM Li or Na salt, 89 mM mannitol, 10 mM Tris-Hepes (pH 7.4), 1 mM $MgSO_4$ and 10 μM taurine. Each point represents the mean ± S.E. of 12 determinations. An asterisk (*) indicates values different from mannitol/NaCl by $p < 0.01$.

TABLE III

EFFECT OF pH AND pH GRADIENT ON TAURINE ACCUMULATION BY BRUSH-BORDER MEMBRANE VESICLES

Brush-border membrane vesicles were preincubated in 287 mM mannitol, 10 mM Tris-Hepes at the pH indicated and 1 mM $MgSO_4$ for 45 min prior to incubation. The incubation mixture included 100 mM NaCl, 87 mM mannitol, 10 mM Tris-Hepes at the pH indicated, 1 mM $MgSO_4$ and 10 μM taurine. n.s., not significant.

pH-in	pH-out	Uptake (pmol/mg protein per 60 s)	
5.5	7.4	27.93 ± 1.70	($n = 12$) n.s.
7.4	7.4	30.10 ± 1.09	($n = 12$) n.s.
5.0	5.0	3.25 ± 0.11 [a]	($n = 12$)
5.5	5.5	3.87 ± 0.32 [a]	($n = 12$)
6.0	6.0	4.36 ± 0.13 [a]	($n = 12$)
6.5	6.5	10.87 ± 0.70 [a]	($n = 12$)
7.0	7.0	26.62 ± 1.43 [a]	($n = 12$)
7.5	7.5	31.80 ± 2.02	($n = 12$)
8.0	8.0	37.42 ± 2.44	($n = 12$)

[a] Different from pH 8.0 by $p < 0.001$.

alter taurine uptake. Thus, an outward directed H^+ ion gradient does not alter taurine accumulation. Preincubation of vesicles at a variety of pH values (using 10 mM Tris-Hepes pH adjusted) followed by incubation with radiolabeled taurine and an external 100 mM NaCl gradient at the same pH does influence uptake. Taurine accumulation is low at pH 5.0 and gradually rises to reach a peak at pH 8.0. Thus, accumulation is favored by a more alkaline pH.

Amino acid inhibition. The effect of other amino acids on taurine accumulation is shown in Table IV. α-Amino acids (at 500 μM) do not alter the uptake of 10 μM taurine over 60 s of incubation. All β-amino acids tested (hypotaurine, β-alanine and β-aminoisobutyric acid) significantly ($p < 0.001$) reduced the uptake of taurine. Hypotaurine and β-alanine (not shown) inhibit taurine uptake in a competitive fashion, as seen in a Dixon plot (Fig. 5) [24]. Taurine thus appears to be transported across the brush border membrane by a β-amino acid group specific mechanism. The K_i of this inhibition is approx. 100 μM. In addition, the accumulation of 10 μM β-alanine uptake was blocked by taurine. Na^+-dependent β-alanine uptake reaches a peak at 6 min (45.3 ± 1.3 pmol/mg protein per 6 min). In the presence of 5.0 mM taurine, this uptake is reduced to 9.1 ± 0.3 pmol/mg protein. Thus, the accumulation of another β-amino acid is blocked by taurine.

Influence of other inhibitors. D-Glucose influences the uptake of taurine as depicted in Fig 6A. Using 5.0 mM D-glucose, the Na^+-dependent uptake of taurine at 10 and 250 μM taurine is reduced over the first six minutes of taurine accumulation. After this time, D-glucose does not influence taurine uptake. As shown in Fig. 6A, in the absence of Na^+, no inhibition of taurine uptake by glucose is evident. Under the conditions of

TABLE IV

Na^+-DEPENDENT TAURINE UPTAKE USING 50 μM TAURINE AND 500 μM OF THE OTHER AMINO ACIDS

Each value is the mean $\pm$ S.E. of 12 determinations.

	Taurine uptake (pmol/mg protein per 60 s)		% of control
	Taurine alone (control)	+ other amino acid	
Methionine	119.92 ± 2.21	99.49 ± 5.06	82.92
Cysteine	137.59 ± 1.95	131.69 ± 9.03	95.71
Leucine	137.97 ± 5.11	128.07 ± 5.16	92.82
α-Amino-isobutarate	93.29 ± 4.74	92.25 ± 3.97	98.88
β-Amino-isobutarate	93.29 ± 4.74	62.88 ± 3.62	67.40
β-Alanine	113.11 ± 4.60	44.11 ± 0.90	39.02
Hypotaurine	113.11 ± 4.60	22.22 ± 0.06	19.64
α-Alanine	137.59 ± 1.95	129.64 ± 5.03	94.22

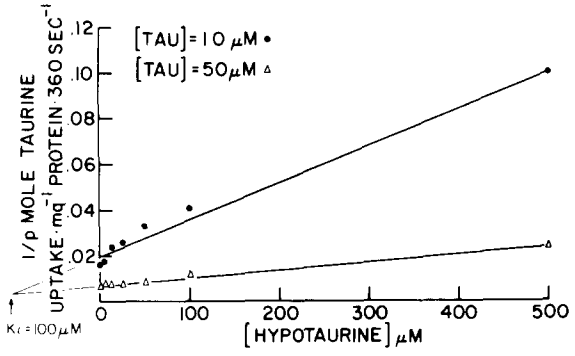

Fig. 5. Dixon plot of taurine uptake in the presence of varying concentrations of hypotaurine (ordinate). Each point represents the mean ± S.E. of 12 determinations.

this experiment (100 mM extravesicular LiCl), D-glucose does not influence taurine accumulation. Accordingly, the inhibition of taurine uptake by D-glucose results from inhibition of the Na^+-dependent taurine transport system. By Dixon plot analysis, glucose inhibits taurine uptake in a competitive fashion with a K_i of 5.5 mM (Fig. 6B).

To further explore this supposed competition of taurine and D-glucose for Na^+, gramicidin (16 μg/ml medium) was added over a wide variety of glucose concentrations (10 μm to 7.0 mM). As shown in Table V, both gramicidin alone and 2.0 mM D-glucose inhibit taurine uptake at two concentrations (10 and 50 μM). The addition of gramicidin to D-glucose always resulted in greater inhibition of uptake than using gramicidin alone or glucose alone.

The transport inhibitor amiloride selectively blocks the Na^+ site of the renal microvillus membrane Na^+/H^+ exchanger [25]. This diuretic does not influence the time course of 10 μM (data not shown) and 250 μM taurine (Fig. 7A). Over the amiloride concentration range 10^{-8} to $9 \cdot 10^{-3}$ M, no influence of amiloride on 10 μM taurine uptake was evident (Fig. 7B). These findings indicate that this diuretic does not inhibit the Na^+-dependent cotransport of Na^+ taurine.

Since amiloride does not influence the sodium-proton exchanged at high sodium concentrations, the accumulation of taurine in a medium containing 10 mM NaCl was examined with and without 9.0 mM amiloride. No influence of amiloride on taurine uptake under the conditions of low NaCl

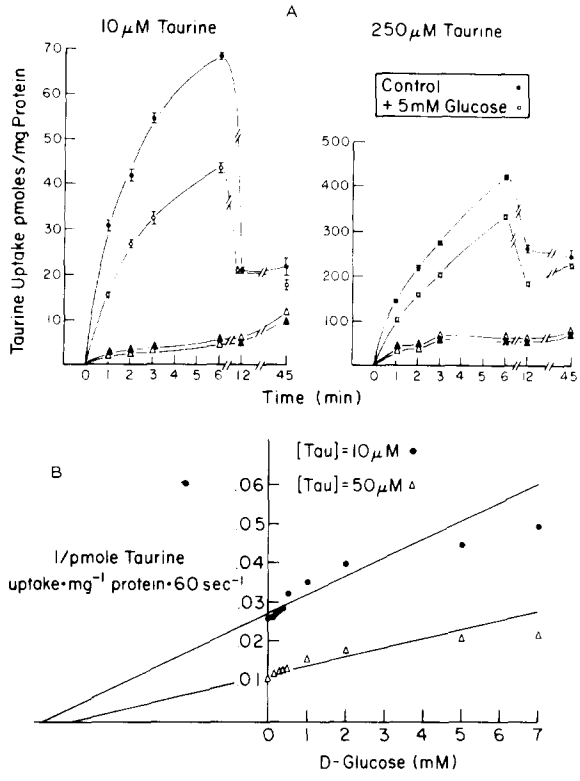

Fig. 6. (A) Na^+-dependent uptake over time of taurine in the presence (○) and absence (●) of 5 mM D-glucose at 10 μM (left) and 250 μM (right) taurine. Each point represents the mean ± S.E. of 12 determinations. (B) Dixon plot of taurine uptake over 60 s in the presence of varying concentrations of D-glucose. The lines are drawn using the least-squares method. Each point represents the mean ± S.E. of 12 determinations.

TABLE V

EFFECT OF GLUCOSE AND GRAMICIDIN ON TAURINE ACCUMULATION

Incubation under usual conditions. Each point is the mean ± S.E. of 12 determinations.

	Taurine uptake (pmol/mg protein per 15 s)	
	10 μM taurine	50 μM taurine
Control	18.3 ±0.60	40.14 ± 4.31
2.0 mM D-glucose	10.98 ± 0.38 [a]	26.55 ± 2.68 [a]
Gramicidin (16 μg/ml)	14.96 ± 0.62 [a]	27.36 ± 1.67 [a]
2.0 mM D-glucose plus gramicidin (16 μg/ml)	8.81 ± 0.33 [a,b]	17.95 ± 0.71 [a,b]

[a] Different from control, $p < 0.01$.

[b] Different from D-glucose alone or gramicidine alone, $p < 0.05$.

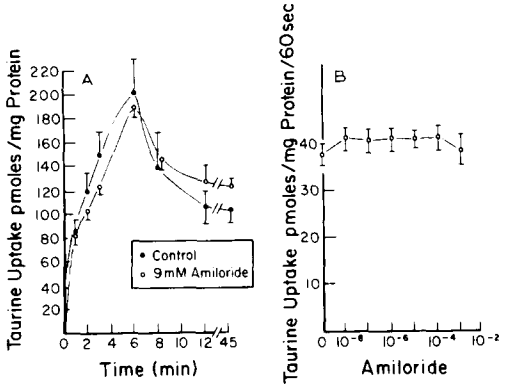

Fig. 7. (A) Taurine (10 μM) uptake over time in the presence (○) and absence (●) of 9 mM amiloride. Each point represents the mean ± S.E. of 12 determinations. (B) Uptake of taurine (10 μM) at 60 s in the presence of varying concentrations of amiloride (10^{-8} to 10^{-3} M). Each point represents the mean ± S.E. of 12 determinations.

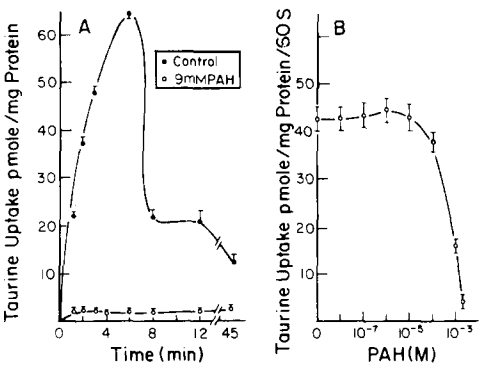

Fig. 8. (A) Uptake of taurine (10 μM) over time in the presence (○) or absence (●) or 9 mM *p*-aminohippurate (PAH). Each point represents the mean ± S.E. of 12 determinations. (B) Taurine (10 μM) uptake by brush-border membrane vesicles in the presence of varying concentrations of PAH (10^{-8} to $9 \cdot 10^{-3}$ M) at 60 s. Each point represents the mean ± S.E. of 12 determinations.

was found (Table VI).

The prototype organic anion, *p*-aminohippurate (PAH) was examined for its influence on taurine accumulation (Fig. 8A, B). *p*-Aminohippurate at 9 mM completely blocks the Na^+-dependent uptake of taurine. However, this inhibition of initial uptake is found only at high concentrations of *p*-aminohippurate, at 10^{-3} M or higher. At 45 min, when equilibrium has been achieved, uptake of taurine is lower in the presence of 9 mM *p*-aminohippurate, potentially indicating an effect independent of the effect on the Na^+-dependent phase (60 s) of taurine accumulation.

TABLE VI

TAURINE UPTAKE AT 10 mM EXTERNAL NaCl, INFLUENCE OF AMILORIDE

Incubation in medium containing 10 mM NaCl, 277 mM mannitol, 10 mM Tris-Hepes (pH 7.4) and 1 mM $MgSO_4$. Each point is the mean ± S.E. of 12 determinations.

Time	Taurine uptake (pmol/mg protein)		
	Control	With 9.0 mM amiloride	*p*
15 s	0.84 ± 0.03	0.77 ± 0.03	n.s.
60 s	1.30 ± 0.06	1.22 ± 0.04	n.s.
6 min	2.89 ± 0.10	2.77 ± 0.11	n.s.
45 min	5.58 ± 0.24	5.62 ± 0.17	n.s.

Discussion

Interaction of taurine with the brush-border surface has been examined in the mouse [6] and rat [7], where accumulation of taurine is sodium-dependent and selective for β-amino compounds. The sodium-dependent K_m for uptake in mouse is 17 μM and 40 μM in rat brush-border membranes. No consistent evidence was found for a second high-K_m, low-affinity uptake site, seen in slices [26,27], since subtraction of that component of uptake accounted for by diffusion alone (uptake in the absence of a NaCl gradient) did not indicate net uptake at taurine concentrations in excess of 500 μM in this study or our previous study [7]. Hammerman and Sacktor [5] examined β-alanine accumulation and also demonstrated a sodium-dependent overshoot for this β-amino acid. Further characteristics of the membrane transport site for β-amino acids have not been well-defined.

Taurine accumulation by vesicles represents uptake rather than binding, since at equilibrium (45 min) the uptake of taurine is inversely related to the osmolarity of the medium when the nonpermeant disaccharide sucrose is employed [7,10]. Extrapolation to infinite osmolarity indicated that membrane binding accounted for less than 5% of the total taurine accumulated. Sonicated membranes took up essentially the same amount of

taurine, indicating minimal binding by two independent methods. The current study independently supports the idea that the uptake of taurine into brush-border membrane vesicles is Na^+-dependent, since mannitol, K^+ or Li^+ did not support an overshoot and since accelerated exchange diffusion of taurine is evident after preloading the membranes with this β-amino acid. If taurine were bound to the membrane, preloading would inhibit the uptake of radiolabelled taurine. Thus, enhanced exchange diffusion can best be explained by transport across a membrane surface [28,29].

The effect of the sodium and potassium ionophores, gramicidin and valinomycin, have been examined [5–7]. Hyperpolarization of the intravesicular space by valinomycin enhances the uptake of taurine and β-alanine after imposition of a potassium outward gradient. Whether the potassium outward gradient alone enhances Na^+-dependent β-amino acid uptake was not addressed by these studies, but an intravesicular greater than extravesicular potassium gradient has been shown to enhance the uptake of L-glutamate, an acidic amino acid [30]. In the current study, we used gramicidin, which dissipates the sodium gradient, and found reduced brush-border membrane vesicle accumulation of taurine. This effect of gramicidin occurs during initial uptake, since no difference in accumulation between gramicidin-treated and non-treated membranes is evident at equilibrium (45 min).

Further insight is gathered from the use of nigericin, where this K^+-H^+ exchange modifier enhances taurine uptake only in the presence of external KCl. Internal KCl or the absence of KCl modifies this nigericin effect, and internal KCl actually results in lower taurine accumulation. This effect of external KCl is not gradient-dependent in that enhanced accumulation is evident where $(KCl)_{in}$ equals $(KCl)_{out}$. To summarize these ionophore effects, taurine uptake is enhanced by internal KCl in the presence of valinomycin, reduced in the presence of gramicidin and enhanced by nigericin with external KCl.

The effects of valinomycin, gramicidin and nigericin may be related to changes in membrane potential. Accordingly, we examined the effect of several anions which are either more (SCN^-, NO_3^-) or less (SO_4^{2-}) permeant than Cl^-. Of all anions examined, the uptake in the presence of Cl^- was greatest. This effect of Cl^- suggested a role for this anion in the activation of the Na^+/taurine cotransport system, as has been described for the Na^+/glycine cotransport systems in teleost and rat vesicles [31,32]. Chloride in these systems stimulates cotransport when placed on the external side. To explore the sidedness of Cl^- influence, vesicles were preloaded with choline chloride, resulting in a 20% reduction in taurine uptake. Choline chloride preincubation studies are potentially marred by the relative impermeability of choline. Thus, both Li^+ and Na^+ salts were used to preload the vesicles. Internal LiCl or NaCl led to a reduction in taurine uptake regardless of the external Li^+ or Na^+ salt used. Preloading with $LiSO_4$ or $LiNO_3$ actually resulted in similar or higher uptake during the overshoot in the presence of NaCl. Hence, the effect of Cl^- appears to be on the external side of the membrane, as has been described in the flounder [33]. The manner in which Cl^- acts is uncertain but may involve some change in membrane confirmation and only acts in conjunction with Na^+.

Existence of a taurine/H^+ antiport system was not found to influence taurine uptake, given the failure of amiloride, even at concentrations as high as $9 \cdot 10^{-3}$ M and in the presence of low external NaCl. Kinsella and Aronson [25] reported no effect of amiloride on either D-glucose or alanine uptake by rabbit brush-border membrane vesicles. Amiloride is felt to be a selective inhibitor for the renal microvillus membrane Na^+/H^+ exchanger [34] and clearly does not alter the Na^+-dependent taurine transport system. Further, taurine uptake in membranes preincubated at pH 5.0, producing an 800 : 1 proton outward gradient, is unaltered. Thus, preloading the vesicle with protons by preincubation in media whose pH is under 7.4 has no influence on taurine uptake despite an H^+-in greater than H^+-out proton gradient which can be transported by a reversible Na^+/H^+ exchanger [35]. However, it should be noted that the electrogenic proton pump in rat renal brush-border membrane vesicles is ATP-driven [36,37]. Since these experiments were carried out in the absence of ATP, some interaction between taurine accumulation and a proton pump cannot be absolutely ruled out.

That other β-amino acids, and not α-amino acids, inhibit the accumulation of taurine is in accord with the existence of a β-amino acid transport system proposed from studies in numerous mammalian species [2–6] including man [38]. This competitive interaction between various β-amino acids is also expressed at the brush border surface in mouse [6] and rabbit [5]. By Dixon plot analysis, hypotaurine inhibits taurine uptake in rat vesicles in a competitive fashion.

The inhibition of the Na^+-dependent component of taurine uptake by D-glucose is similar to the interaction between glucose, alanine and phosphate described by Barrett and Aronson [39]. They concluded that alanine and glucose inhibited phosphate as an indirect effect of solute-induced changes in the transmembrane electrochemical Na^+ gradient rather than as a result of any competition for a carrier that could transfer these solutes or from allosteric interactions. It is likely that glucose and taurine compete for the Na^+ gradient, particularly since D-glucose and taurine, which have no structural similarities, appear to exhibit competitive inhibition. The D-glucose inhibitory effect is seen after 15-s incubation (initial rate) and the addition of gramicidin, which dissipates the Na^+ gradient, further blocking taurine uptake. The finding of additive effects of D-glucose and gramicidin indicates that interaction with Na^+, an indirect effect on taurine transport, is more likely than a direct interaction of D-glucose with the taurine transport system, since gramicidin affects the Na^+ gradient. The absence of any effect of glucose on taurine in the presence of a 100 mM LiCl gradient further indicates this competition for the Na^+ gradient.

p-Aminohippurate is considered to be a prototype substance for the weak organic anion secretory system. Since *p*-aminohippurate is actively accumulated by cortex slices and by rabbit kidney basolateral membrane vesicles [41,42], the basolateral surface appears to be the principal location of *p*-aminohippurate transport. Sodium-energized *p*-aminohippurate transport is found only in basolateral membranes, since no 'overshoot' is found when brush-border vesicles are used [41]. We find that *p*-aminohippurate, at concentrations at or exceeding 10^{-3} M, will inhibit taurine accumulation by brush-border membrane vesicles. These results suggest some interaction between taurine uptake and a compound transported by the organic anion transport mechanism. However, even at 100-fold higher concentrations of *p*-aminohippurate, taurine uptake is inhibited by only 70%, and complete inhibition is found using 9 mM *p*-aminohippurate, a 9000-fold higher level of inhibitor.

In conclusion, taurine is taken up by brush-border membrane vesicles by a Na^+-dependent, β-amino-acid-specific transport system. Gramicidin impairs taurine uptake, but no effect of amiloride or an intravesicular-to-extravesicular proton gradient is found. D-Glucose at high levels blocks taurine uptake, possibly by competition for a Na^+ gradient. Some interaction between taurine uptake and the weak organic anion transport mechanism is indicated by PAH inhibition, albeit at very high inhibitor concentrations. Finally, taurine uptake is maximal when Cl^- is the anion used and present on the external surface of the vesicle, since internal Cl^- actually lowers taurine uptake.

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References

1 Dantzler, W.H. and Silbernagl, S. (1976) Pfluegers Arch. 357, 123–128
2 Chesney, R.W., Scriver, C.R. and Mohyuddin, F. (1976) J. Clin. Invest. 57, 183–193
3 Chesney, R.W. and Jax, D.K. (1977) Biochim. Biophys. Acta 466, 84–96
4 Friedman, A.L., Albright, P.W., Gusowski, N., Padilla, M. and Chesney, R.W. (1983) Am. J. Physiol. 245, F159–F166
5 Hammerman, M. and Sacktor, B. (1978) Biochim. Biophys. Acta 509, 338–347
6 Rozen, R., Tenenhouse, H.S. and Scriver, C.R. (1979) Biochem. J. 180, 245–248
7 Chesney, R.W., Gusowski, N. and Friedman, A.L. (1983) Kidney Int. 24, 588–564
8 Rozen, R. and Scriver, C.R. (1982) Proc. Natl. Acad. Sci. USA 79, 2101–2105
9 Booth, A.G. and Kenny, A.J. (1974) Biochem. J. 142, 575–581

10 Chesney, R.W., Friedman, A.L., Albright, P.W. and Gusowski, N. (1982) Proc. Soc. Exp. Biol. Med. 170, 493–501
11 Glossman, H. and Neville, D.M., Jr. (1972) FEBS Lett. 19, 340–344
12 Dixon, T.F. and Purdom, M. (1954) J. Clin. Pathol. 7, 341–343
13 Post, R.L. and Sen, A.K. (1981) in Methods in Enzymology (Estabrook, R.W. and Pullman, M.E., eds.), Vol. 10, pp. 762–768, Academic Press, New York
14 Lowry, O.H. and Passonneau, J.V. (1972) A Flexible System of Enzymatic Analysis, pp. 201–204, Academic Press, New York
15 Sottocasa, G.L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) J. Cell. Biol. 32, 415–428
16 Koldovsky, O. (1971) Arch. Biochem. Biophys. 142, 378–381
17 Kunin, C.M. and Chesney, R.W. (1978) Pediatrics 62, 751–762
18 Burton, K. (1956) Biochem. J. 62, 315–322
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
20 Chesney, R.W., Sacktor, B. and Rowen, R.R. (1973) J. Biol. Chem. 218, 2182–2191
21 Neal, J.L. (1972) J. Theor. Biol. 35, 113–118
22 Schneider, E.G., Hammerman, M.R. and Sacktor, B. (1980) J. Biol. Chem. 255, 7650–7656
23 Singer, I. and Civan, M.M. (1971) Am. J. Physiol. 221, 1019–1026
24 Neame, K.D. and Richards, T.G. (1972) Elementary Kinetics of Membrane Carrier Transport, pp. 56–79, John Wiley & Sons, New York
25 Kinsella, J.L. and Aronson, P.S. (1981) Am. J. Physiol. 241, F374–F379
26 Chesney, R.W. and Jax, D.K. (1978) in Taurine in Neurological Disorders (Huxtable, R. and Barbeau, A., eds.), pp. 73–94, Raven Press, new York
27 Chesney, R.W. and Jax, D.K. (1979) Pediatr. Res. 13, 854–860
28 Rosenberg, T. and Wilbrandt, W. (1957) J. Gen. Physiol. 41, 289–296
29 Lieb, W.R. and Stein, W.D. (1972) Biochim. Biophys. 265, 187–207
30 Schneider, E.G. and Sacktor, B. (1980) J. Biol. Chem. 255, 7645–7649
31 King, P.A., Beyenbach, K.W. and Goldstein, L. (1982) J. Exp. Zool. 223, 103–114
32 Boge, G. and Rigal, A. (1981) J. Biol. Chem. 16, 455–461
33 Corcelli, A. and Storelli, C. (1983) Biochim. Biophys. Acta 732, 24–31
34 Aronson, P.S. and Kinsella, J.L. (1981) Fed. Proc. 40, 2213–2217
35 Schwartz, G.J. (1981) Am. J. Physiol. 241, F380–F385
36 Murer, H., Kinne-Saffran, E., Beauwens, R. and Kinne, R. (1980) Proc. 27th Int. Congr. Physiol. Sci., pp. 200–201, Hungarian Physiological Society, Budapest
37 Kinne-Saffran, E., Beauwens, R. and Kinne, R. (1982) J. Membrane Biol. 63, 67–76
38 Goldman, H. and Scriver, C.R. (1967) Pediatr. Res. 1, 212
39 Scriver, C.R., Nutzenadel, W. and Perry, T.L. (1978) in The Metabolic Basis of Inherited Disease (Stanbury, J.B., Wyngaarden, J.B. and Fredericksen, D.S., eds.), 4th Edn., pp. 528–542, McGraw-Hill, New York
40 Barrett, P.Q. and Aronson, P.S. (1982) Am. J. Physiol. 242, F126–F131
41 Sheikh, M.I. and Moller, J.V. (1982) Biochem. J. 208, 243–246
42 Cross, R.J. and Taggart, J.V. (1950) Am. J. Physiol. 161, 181–190