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Sodium-taurine cotransport in reptilian renal brush-border membrane vesicles

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Abstract. The coupled transport of Na^+ with taurine into snake renal brush-border membrane vesicles (BBMV) was studied using 5-s uptake conditions. Taurine transport into snake renal BBMV involved two parallel processes, one saturable (Na^+-dependent) and one (Na^+-independent) that behaved like passive diffusion. Below 1 mM taurine concentration, the Na^+-dependent system accounted for 60% of total taurine uptake. Over both low (0.001 – 0.80 mM) and high (0.8 – 5.0 mM) taurine concentration ranges, the Na^+-dependent taurine uptake within each range showed Michaelis-Menten kinetics, suggesting the presence of two independent saturable Na^+-dependent transport systems for taurine. The high-affinity, low-capacity system saturated above 100 μM with a K_m of 71.4 ± 45.7 μM and a maximum velocity (V_{max}) of 21.9 ± 3.77 pmol (mg protein)$^{-1}$ (5 s)$^{-1}$. The low-affinity, high-capacity system saturated above 1 mM, with a K_m of 1.11 ± 0.63 mM and a V_{max} of 252 ± 47 pmol (mg protein)$^{-1}$ (5 s)$^{-1}$. The stoichiometric relationship between external Na^+ concentration and taurine uptake (at 10 μM) by the high-affinity BBMV transport system was examined by the activation method under short-circuited conditions. The 5-s rate of taurine transport was a sigmoid function of increasing extravesicular Na^+ concentration. Kinetic analysis of the interaction of Na^+ with the high-affinity taurine transport system suggested that 3 Na^+ ions (3.2 ± 0.7) may be involved with 1 taurine molecule in the transport event. The data suggest the presence of a highly efficient and high-affinity reabsorptive taurine transport system on the luminal membrane of the kidney of the garter snake, a species that can secrete taurine in vivo.

Key words: Taurine – Kinetic – Stoichiometry – Amino acid transport – β-Amino acid – Membrane vesicle – Kidney – Sodium-coupled transport

Introduction

Taurine, a ubiquitous amino acid, can either be reabsorbed or secreted by the renal tubules of vertebrate kidneys. Mammalian kidneys physiologically exhibit only net reabsorption of taurine, whereas the kidneys of marine teleost fishes exhibit only net secretion of the amino acid [15]. Ophidian reptiles, on the other hand, demonstrate both net reabsorption and net secretion of taurine in vivo under physiological conditions [1, 2]. The renal brush-border membranes of vertebrate proximal tubule cells contain a transport system for taurine, the general chracteristics of which appear to be well-conserved during the course of the evolution of vertebrate kidneys [4]. Despite the observed differences in the direction of net transport of taurine in vivo, the renal brush-border transport systems for taurine in the three vertebrate classes similarly involve an electrogenic secondary active cotransport of Na^+-Cl^--taurine [4, 17, 27, 29, 30]. In mammalian kidneys, which only exhibit net reabsorptive transport of taurine, the stoichiometry for this brush-border transport system appears to be 2 Na^+:1 Cl^-:1 taurine [27, 30]. Na^+ facilitates the interaction of taurine with the carrier and its transmembrane gradient provides the driving force for the uphill transport of the amino acid [21, 29]. The anion Cl^- is thought to play a role in the translocation process by being co-transport with the Na^+-taurine complex [27, 30]; in some cases a Cl^- gradient could also energize the concentrative taurine uptake [31].

In the kidneys of nonmammalian vertebrates that can secrete taurine, namely fishes and ophidian reptiles, the nature of the interaction between Na^+ and taurine and the stoichiometric coupling of the renal brush-border taurine cotransport system have not been reported. The transport stoichiometry will have significant implications for the energetics of taurine transport and the orientation of the carrier. In the flounder renal tubule, which secretes taurine, it has been hypothesized that because of the high taurine concentration found in the renal cells of this species, the Na^+-taurine cotransporter in the luminal membrane could operate in a reverse direction with the stoichiometry of 1 Na^+:1 taurine, producing net efflux of the amino acid from the renal cell into the tubular lumen [17]. However, a coupling stoichiometry between Na^+ and taurine of greater than one would impart a

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thermodynamic barrier for the operation of the flounder taurine transport system on the luminal membrane in the secretory direction. In addition, since there are sodium-dependent taurine transport systems on both brush-border [17] and basolateral membranes [16] of the flounder renal cells, net secretion of taurine can be manifested only if (a) the Na^+-taurine cotransport system on the basolateral membrane has a higher coupling stoichiometry than the Na^+-taurine cotransport system in the luminal membrane [18] or, (b) the kinetics of the luminal taurine transport system is such that it is of sufficiently low capacity to allow secretion to occur via a different pathway. The potential importance of the transport kinetics and the coupling stoichiometry on the net direction of taurine transport across the renal cell has led us to examine the kinetic characteristics of Na^+-taurine cotransport in the renal brush-border membrane vesicles (BBMV) of the garter snake kidney, a species that can secrete taurine in vivo [2, 4]. The stoichiometric coupling between Na^+ and taurine of this transport system was also evaluated. Our findings indicate an involvement of multiple Na^+ ions with a high-affinity luminal taurine transport system in this secretory species, a condition highly favorable for taurine reabsorptive flux across the luminal membrane. This suggests that other luminal transport mechanism(s) might be responsible for taurine secretion in this vertebrate kidney.

Materials and methods

Animals. Adult garter snakes (*Thamnophis sirtalis*) of both sexes were used in the present study. They were obtained from commercial suppliers in Wisconsin and maintained in glass aquaria at 25° C. The snakes were fed regularly with a diet of raw fish and earthworms supplemented with vitamins and minerals and had free access to water.

Preparation of renal brush-border membrane vesicles. For a preparation of snake renal BBMV, six to ten garter snakes were decapitated and their kidneys quickly excised. The renal brush-border membranes were prepared from kidney homogenates by a combination of hypotonic lysis, Ca^{2+} precipitation, and differential centrifugation as described in Benyajati and Dantzler [3] with the following modifications. Homogenization was performed with a Tissumizer (24000 rpm, Tekmar) at 45% power setting for 10 s. Two precipitation steps were performed with 30 mM $CaCl_2$. For each step, the $CaCl_2$-treated supernatant was stirred for 15 min at 4 °C. The slow-speed centrifugations to sediment aggregated material were at 3000 *g* and 5900 *g* for 10 min, and the higher-speed brush-border membrane collection was at 32800 *g* for 20 min. Purified membranes were suspended in 300 mM mannitol, 1 mM HEPES/TRIS (pH 7.5) at a protein concentration of 4–5 mg/ml. The final brush-border membrane suspension was routinely enriched 12- to 15-fold with respect to the specific activity of the brush-border marker, alkaline phosphatase, compared to the initial homogenate. The basolateral marker, K^+ *p*-nitrophenol phosphatase, was only slightly enriched (2-fold), and the specific activity of the mitochondrial marker, succinate dehydrogenase, was reduced at least 2-fold. The extent of cross-contamination of the isolated brush-border membranes with the basolateral membranes was quantitatively determined by the method of Cheung and Hammerman [8], using absolute activities of marker enzymes from concurrent preparations of snake renal brush-border and basolateral membranes. The cross contamination of our snake brush-border membrane preparations by basolateral membranes was usually about 15%. Protein was measured by a Coomassie blue protein assay (Bio-Rad) using bovine serum gammaglobulin as the standard. Aliquots of freshly prepared vesicles were fast-frozen and stored in liquid N_2 until use (usually within 3–4 days). Storage over this period did not affect the viability of the vesicles as measured by the rates of solute uptake [3, 4].

Transport studies with membrane vesicles. Uptake of [^{3}H]taurine into membrane vesicles was measured by a rapid filtration technique [4], utilizing an automated rapid-uptake apparatus (Short-time Reaction/Uptake Measurement Apparatus; Innovativ Bischoff AG, Wallisellen, Switzerland) that allows automatic control of incubation times as short as 0.2 s. A 90-µl aliquot of transport buffer containing approximately 10 µCi/ml [^{3}H]taurine and appropriate salt concentrations (the composition of the incubation media and manipulations of the vesicles are described in the figure legends) was rapidly mixed with a 10-µl aliquot of the membrane suspension, the mixing being accomplished by a solenoid vibrator. The transport reaction was carried out at room temperature (25 ± 2° C). At an electronically preset time the reaction was automatically stopped, 1 ml ice-cold stop solution was injected into the reaction tube using compressed air controlled by an electromagnetic valve. Stop solutions were of identical composition to the incubation media but contained no substrates. A 900-µl aliquot of the mixture of membranes and stop solution was filtered through a pre-wetted 0.45 µm nitrocellulose filter (Millipore). The filter was washed with 4 ml stop solution and placed in a scintillation vial for determination of radioactivity. Since low passive permeability of the snake renal BBMV to taurine has previously been demonstrated [4], the loss of intravesicular labelled taurine during the stopping and washing procedure should be negligible. Preliminary experiments confirmed that there was no significant leakage of labelled taurine in the stop solution during the 10-s stopping and washing process. Nonspecific binding of substrate by the membranes and filters (zero-time uptake) was determined and corrected for each set of samples by adding the stop solution to the transport buffer and membrane suspension before the latter two were mixed. Earlier studies indicate that in snake renal BBMV, the taurine uptake measurement corrected for the zero-time uptake adequately corrects for membrane binding and represents only the transport component of taurine into intravesicular space [4]. Uptake into the vesicles was usually expressed as picomoles of substrate accumulated per milligram of membrane protein. Measurements at all experimental points were performed in triplicate using membranes that were either prepared on the same day or previously stored in liquid N_2. Each experiment was repeated at least three times on different membrane preparations.

Estimation of uptake kinetics and stoichiometry. For the analysis of kinetic characteristics of taurine transport, 5-s uptakes were considered to be reasonable estimates of the initial rates of taurine transport (see justification below). Previous studies showed that taurine uptake into snake renal BBMV exhibits electrogenicity [4]. Therefore for stoichiometric studies, the electrical effect of a transmembrane Na^+ gradient on taurine uptake was minimized by clamping the vesicle electrical potential at an inside-negative K^+-diffusion potential using a valinomycin/K^+ voltage clamp. The BBMV were preloaded with 100 mM KCl and preincubated for 30 min at 25° C with valinomycin (25 µM/mg protein). Taurine uptake was measured in the buffer containing valinomycin but no KCl.

Kinetic parameters for the observed BBMV taurine uptake data were estimated by a computer curve-fitting procedure, using an iterative, non-linear method (SigmaPlot Scientific Graph System, Jandel Scientific), fitting the uptake data to the appropriate kinetic equations (Michaelis-Menten or Hill). The non-linear curve-fitting procedure yields a best fit with the least biasing of the raw data; in addition estimates of the variances can also be obtained.

Statistical analysis. Data are expressed as means (±SE) of the number of experiments indicated by *n*, with the exception of the estimates of the kinetic parameters where data are expressed as estimates ± SD.

Chemicals. Radiolabelled [1,2^{-3} H]taurine (35 Ci/mmol) was obtained from Amersham. All other chemicals were purchased from Sigma Chemical Co. and were of the highest purity available.

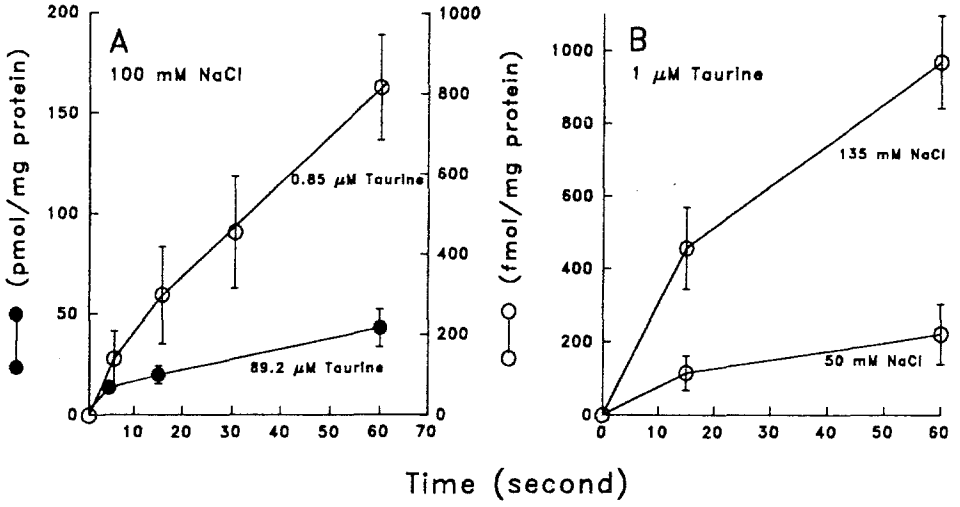

Fig. 1A, B. Time course of brush-border membrane vesicle (BBMV) Na^+-dependent taurine uptake. Vesicles were prepared in 300 mM mannitol, 1 mM HEPES/TRIS, pH 7.5. Uptake was measured in presence of 100 mM mannitol, 1 mM HEPES/TRIS (pH 7.5) and, varying concentrations of taurine (A) and Na^+ (B) as indicated in the figure. Na^+-dependent taurine uptake values were calculated by subtracting taurine uptake in mannitol buffer (300 mM) from that in presence of Na^+. Values and means ±SE of two to five paired experiments, each carried out in triplicate. ○, Taurine uptake in fmol/mg protein; ●, in pmol/mg protein

Results

Initial time course of Na^+-dependent taurine uptake into snake renal BBMV

The first 60 s of Na^+-dependent taurine uptake into renal brush-border membranes is shown in Fig. 1. At a given taurine concentration, the magnitude of BBMV taurine transport is the sum of Na^+-dependent and -independent components of the transport (see Fig. 2). Although snake renal BBMV taurine transport is dependent on both Na^+ and Cl^- [4], only the effect of Na^+ dependence was examined in the present study. The Na^+-dependent taurine uptake shown in Fig. 1 was obtained by subtracting the uptake in mannitol buffer (Na^+-independent uptake) from the paired uptake measured in the presence of 100 mM NaCl in the external medium. Taurine uptake was non-linear over the concentration ranges of taurine (Fig. 1A) and NaCl (Fig. 1B). In the present study, the 5-s uptake values were employed for the kinetic and the stoichiometric studies. Since the rate of snake renal BBMV taurine uptake was very low (mostly in the fmol/mg protein range), the 5-s uptake values were the earliest time points that could actually be reliably measured with accuracy and reproducibility.

To evaluate whether the 5-s uptake values for taurine could be used as meaningful estimates for the intial rates in this particular study, the method for determining initial velocities of Cornish-Bowden [9] was employed. The Cornish-Bowden method utilizes the integrated form of the Michaelis-Menten equation to estimate the initial velocity of an enzyme-catalysed reaction (or in this case a carrier mediated transport), using the data on the measurements of product concentrations at several time points (i.e. the progress curve). The method is appropriate for systems in which the initial straight part of the reaction is very short or non-existent. More importantly, the method does not require precise knowledge of the reaction at longer time points or at equilibrium. By applying the Cornish-Bowden method to the Na^+-dependent taurine-uptake data from 5 s up to 2 h, the initial rate of BBMV uptake of taurine (1 μM) was estimated to be 33 fmol/mg protein (actual analysis not shown), a value not significantly different from the taurine uptake rate experimentally measured at 5 s (mean of three experiments = 28.1 ± 13.7 fmol/mg protein). Therefore, based on the Cornish-Bowden analysis we considered the 5-s uptake data in the present study to be rasonable and meaningful estimates for the initial rates of taurine uptake into snake renal BBMV.

It should be noted that the 5-s uptake in the present study was the earliest time point used in kinetic studies of taurine transport in any vertebrate kidney; uptake values at 15–30 s were used in other renal membrane vesicle studies (see Table 1).

Kinetic characteristics of Na^+-dependent taurine transport in snake renal BBMV

The effect of increasing taurine concentration (1 μM to 5 mM) on the rate of brush-border taurine uptake (5 s) in the presence and absence of an inwardly directed 100 mM NaCl gradient is illustrated in Fig. 2. At a given concentration of taurine, BBMV uptake of taurine (open circles) reflected the sum of contributions from Na^+-dependent (filled circles) and -independent (open triangles) transport systems. The Na^+-independent taurine transport (dotted line) did not saturate even at 5 mM taurine concentration. Below 1 mM, the Na^+-independent system accounted for approximately 40% of the total taurine transport across snake renal brush-border membranes: at 90 μM taurine, Na^+-independent taurine transport was 8.9 ± 3.5 pmol (mg protein)$^{-1}$ (5 s)$^{-1}$ and the total taurine transport was 22.4 ± 6.6 pmol (mg protein)$^{-1}$ (5 s)$^{-1}$; at 450 μM taurine, Na^+-independent taurine transport was 13.1 ± 1.5 pmol (mg protein)$^{-1}$ (5 s)$^{-1}$ and the total taurine transport was 34.5 ± 8.3 pmol (mg protein)$^{-1}$ (5 s)$^{-1}$. However, above 1 mM taurine concentration, the Na^+-independent transport system accounted for most of the brush border taurine uptake.

With increasing taurine concentrations over a very wide range (0.001–5.0 mM), the rate of Na^+-dependent taurine transport (solid line, Fig. 2) increased as if there were two independent saturable transport systems for taurine in the snake renal brush-border membrane, one operating at taurine concentrations in the micromolar range and the other operating in the millimolar range.

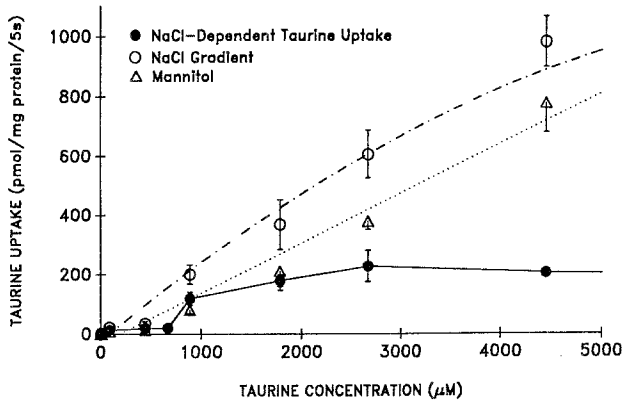

Fig. 2. Effect of increasing taurine concentration on the rate (5 s) of BBMV taurine uptake. Vesicles contained 300 mM mannitol, 1 mM HEPES/TRIS, pH 7.5. Transport buffers contained either 100 mM NaCl + 100 mM mannitol or 300 mM mannitol, 1 mM HEPES/TRIS (pH 7.5), with tracer ([1,2-^{3}H]taurine), and increasing concentrations of unlabelled taurine from 0.001 mM to 5.0 mM (final concentration). Each point represents mean ± SE of two to four paired experiments with and without NaCl gradient. Each experiment was carried out in triplicate using different membrane preparations. In those cases where *vertical bars* are absent, SE are smaller than the graphical representations of the means. The *solid line*, drawn from point to point, represents the mean Na^+-dependent taurine uptake obtained from the paired experiments and was obtained by subtracting the uptake in mannitol buffer from the paired uptake in the presence of 100 mM NaCl at each concentration of taurine

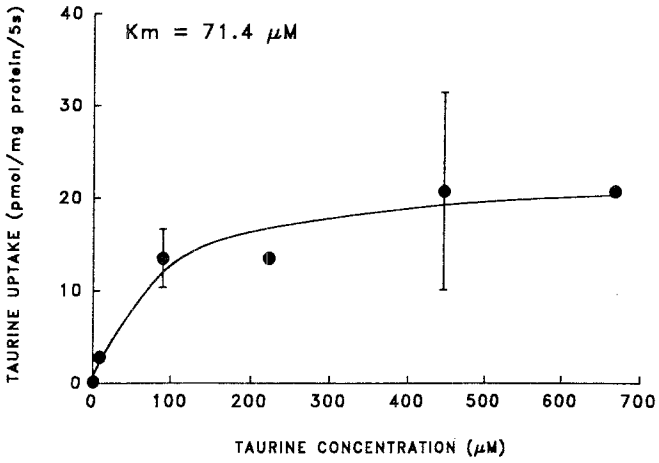

Fig. 3. Na^+-dependent taurine uptake at low taurine concentrations (1 – 700 μM). The experimental conditions were similar to those in Fig. 2. Values are means ± SE of two to four paired differences between taurine uptake in 100 mM NaCl and in 300 mM mannitol buffers. In those cases where *vertical bars* are absent, SE are smaller than the graphical representations of the means. The curve was fitted to the Michaelis-Menten equation by an iterative non-linear method. K_m = 71.4 ± 45.7 μM, V_{max} = 21.9 ± 3.77 pmol(mg protein)$^{-1}$ (5 s)$^{-1}$, r = 0.783

In the low micromolar range of taurine concentration (0.001 – 0.8 mM), Na^+-dependent taurine uptake approached saturation at approximately 100 μM (see Fig. 3). At taurine concentrations above 0.8 mM, there was a sharp rise in the rate of taurine uptake as if another transport system for taurine with much higher transport

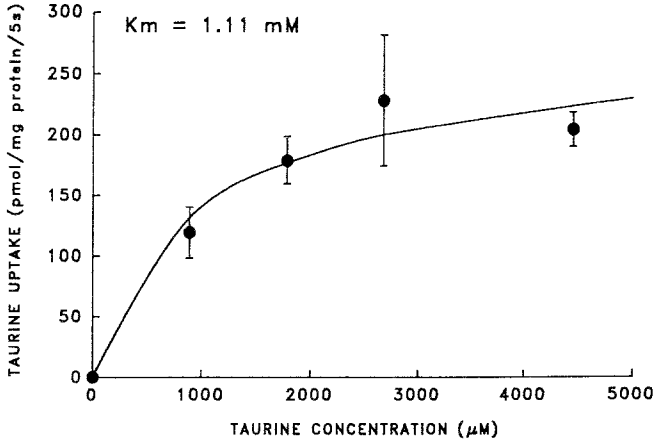

Fig. 4. Na^+-dependent taurine uptake at high taurine concentrations (0.7 – 5 mM). The experimental conditions were similar to those in Fig. 2. Values are means ± SE of two to four paired differences between taurine uptake in 100 mM NaCl and in 300 mM mannitol buffers. The curve was fitted to the Michaelis-Menten equation by an iterative non-linear method. The K_m value was estimated after correction for the contribution of the high-affinity taurine transport system. K_m = 1.11 ± 0.63 mM, V_{max} = 252 ± 47 pmol(mg protein)$^{-1}$ (5 s)$^{-1}$, r = 0.881

rates was switched on. This latter system approached saturation at taurine concentrations above 1 mM (see Fig. 4).

The kinetics of taurine uptake over the whole range of taurine concentrations examined (0.001 – 5.0 mM) was rather complex and could not be satisfactorily fitted with a Hill-type equation or a modified Michaelis-Menten equation [25] that takes into account the contribution of two (overlapping) saturable transport systems. In order to simplify the analysis of the kinetics of the BBMV taurine transport without going into more complicated kinetics models, we analysed the Na^+-dependent taurine uptake data over the two concentration ranges separately. It was assumed that the snake renal BBMV contained two independent transport systems for taurine that did not contribute appreciably to the observed transport rate at the taurine concentration outside its range. This assumption was based on the observed sharp rise in the rate of BBMV taurine uptake at taurine concentrations above 0.8 mM, which could suggest the possible existence of a taurine transport system that operated only at high taurine concentrations. Therefore, the interpretation of the kinetics of taurine uptake in the snake renal BBMV should be considered in light of this simplification.

On the basis of the above assumption, the Na^+-dependent taurine uptake was examined over the low (0.001 – 0.8 mM) taurine concentration range (Fig. 3), separately from the high concentration range (0.8 – 5.0 mM; Fig. 4). The raw experimental points, below taurine concentration of 800 μM (data of Fig. 3) and above taurine concentration of 800 μM (data of Fig. 4), were fitted separately, each with a best-fitting curve by the iterative non-linear method. Each set of data was curve-fitted to the Michaelis-Menten equation for saturation kinetics: $v = V_{max}[S]/(K_m + [S])$. v is BBMV taurine uptake in pmol(mg protein)$^{-1}$ (5 s)$^{-1}$, [S] is the taurine

concentration in μM, V_{max} is the maximal rate of BBMV taurine uptake, and K_m is the concentration of taurine that yields one-half V_{max}. The kinetic parameters for the two Na^+-dependent taurine transport systems were estimated from the best-fitting curves. From Figs. 3 and 4 it can be seen that within each range the Na^+-dependent taurine uptake could satisfactorily be described by a Michaelis-Menten type kinetics (regression coefficients for both ≥ 0.8). The estimated kinetic parameters for the BBMV Na^+-dependent taurine transport system in the low taurine concentration range (high-affinity, low-capacity system) were as follows: $K_m = 71.4 \pm 45.7$ μM and $V_{max} = 21.9 \pm 3.77$ pmol(mg protein)$^{-1}$ (5 s)$^{-1}$ (Fig. 3). For the BBMV Na^+-dependent taurine transport system in the high taurine concentration range (low-affinity, high-capacity system), $K_m = 1.11 \pm 0.63$ mM and $V_{max} = 252 \pm 47$ pmol(mg protein)$^{-1}$ (5 s)$^{-1}$ (Fig. 4). The kinetic estimates for the low-affinity system were corrected for the activity of the high-affinity component, because the taurine uptake values at high taurine concentrations may represent both the high-affinity and the low-affinity transport whereas at low taurine concentrations only the high-affinity system predominates.

Stoichiometry of Na^+-taurine cotransport in snake renal brush-border membranes

It has been shown in earlier studies [4] that snake renal BBMV taurine uptake is specifically dependent on the presence of an inwardly directed Na^+ gradient. To elucidate the nature of the Na^+ requirement further, we investigated the stoichiometric relationship between external Na^+ concentration and the taurine uptake (10 μM) by the high-affinity BBMV taurine transport system under short-circuited zero-trans Na^+ and taurine conditions. Na^+ concentration gradients across the membranes were varied from 0 to 150 mM, using choline to replace Na^+ isosmotically. Since snake renal BBMV taurine uptake is also dependent on the presence of the anion Cl^- and is stimulated by an inwardly directed Cl^- gradient at 5 s [4], Cl^- concentrations were maintained constant on both sides of the membrane at 100 mM. This allowed the determination of the effect of the Na^+ gradient on BBMV taurine transport independently of the possible influence of a Cl^- gradient. The results are shown in Fig. 5. Increasing the concentration of Na^+ in the extravesicular medium produced a sigmoid stimulation in the rate of taurine transport. The sigmoid relationship suggests that more than one Na^+ ion is associated with the taurine transport event [26]. The most common method for estimating the number of Na^+ ions involved in the transport process is based on analysis using the Hill equation for multiple substrate/activator reactions [23]. The data for the interaction of Na^+ and taurine transport in Fig. 5 were therefore fitted to the Hill equation:

$$J = \frac{J_{max}\,[Na^+]^h}{K' + [Na^+]^h}$$

where K' is a constant comprising the interaction factors between binding sites and the intrinsic association constants, and h is the Hill coefficient, an estimate of the number of Na^+-binding sites. In the present experimental conditions, the computer analysis yielded an estimate of three Na^+ ions (3.2 ± 0.7) participating in the uptake of taurine across snake renal brush-border membrane (regression coefficient = 0.996), with a J_{max} for 10 μM taurine uptake of 2432 ± 155 fmol(mg protein)$^{-1}$ (5 s)$^{-1}$ and a mean affinity constant for Na^+ (the Na^+ concentration at half-maximal transport rate) of 49.7 mM.

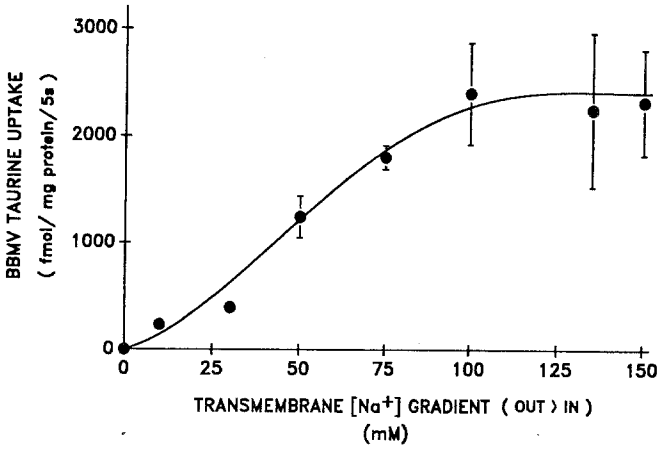

Fig. 5. Effect of increasing extravesicular Na^+ concentrations on BBMV taurine transport. Vesicles were pre-equilibrated with 100 mM mannitol, 100 mM KCl, 1 mM HEPES/TRIS, pH 7.5, and 25 μM valinomycin/mg protein (0.1 mM solution). Transport buffers contained 10 μM [^{3}H]taurine, 1 mM HEPES/TRIS (pH 7.5), 120 μg/ml valinomycin (0.1 mM), and varying concentrations of NaCl (from 0 to 150 mM). Isosmolality was maintained with choline chloride. Final ethanol concentration in all vesicles was less than 1%. Incubation times were 5 s data represent only the Na^+-dependent component of taurine uptake. Data points are means ± SE of three to five experiments, each carried out in triplicate using different membrane preparations. In those cases where *vertical bars* are absent, SE are smaller than the graphical representations of the means. The curve was fitted to the Hill equation by an iterative, non-linear method. For 10 μM taurine, J_{max} was 2.4 ± 0.16 pmol(mg protein)$^{-1}$ (5 s)$^{-1}$, K_{Na} for Na^+ was 50 mM, and the apparent Hill coefficient, h, was 3.2 ± 0.7; $r = 0.996$

Discussion

The present study is the first kinetic characterization of the luminal renal taurine transport system in a species that can secrete taurine under physiological conditions. Our data indicate that taurine was transported across the apical membrane of the snake proximal renal cells by both Na^+-independent and Na^+-dependent processes. At in vivo plasma concentrations of taurine in the garter snake (≈ 1 μM, [1]), the Na^+-dependent taurine transport system accounted for approximately 60% of the total apical taurine uptake. Two saturable Na^+-dependent transport systems with different affinities for taurine were demonstrated for taurine uptake across the renal brush-border membrane of the snake: a high-affinity, low-capacity system with an estimated K_m for taurine of 70 μM and a low-affinity, high-capacity system with a K_m of 1.1 mM. The presence of two saturable transport systems for taurine on the luminal membrane of snake

renal epithelium and their estimated K_m values are in good agreement with the recent findings in rabbit proximal tubule [14]. In both pars convoluta and pars recta of the rabbit kidney, the uptake of taurine by luminal membrane vesicles was characterized by two Na^+-dependent processes: high-affinity (K_m = 86 μM for pars convoluta and 12 μM for pars recta) and low-affinity (K_m = 5.4 mM for pars convoluta and 5.6 mM for pars recta) systems (see Table 1). Previously, only one saturable and high-affinity Na^+-dependent taurine transport system (K_m range = 17–40 μM) has been described for the uptake of taurine across the renal brush-border membrane vesicles of mammalian kidneys (Table 1).

For the snake renal BBMV, it is interesting to note that at low taurine concentrations (<0.8 mM), Na^+-dependent taurine uptake appeared to be mediated only by the high-affinity system, whereas at high taurine concentrations (0.8–5.0 mM) the uptake values probably reflected both the high-affinity and low-affinity transport of taurine. This inference was made from the observed sharp rise in the rate of BBMV taurine uptake at taurine concentrations above 0.8 mM (Fig. 2), which could not be explained on the basis of the overlapping presence of two saturable transport systems over the whole taurine concentration range examined. If the low-affinity taurine transport system were operative at low taurine concentrations (<0.8 mM), its contribution to the overall taurine uptake in that range would be increasing with taurine concentrations. However, the actual measured rates of taurine uptake over this range reached a plateau at about 21 pmol(mg protein)$^{-1}$ (5 s)$^{-1}$, a rate significantly lower than the expected total uptake that included contributions from the low-affinity system. Indeed, careful inspection of the data from the rabbit renal BBMV (Fig. 2 of [14]) also revealed similar observations: the actual taurine transport rates in the low concentration range were smaller than the expected contributions from both high-affinity and low-affinity systems. The presence of a low-affinity transport system for taurine that is only operative in the high concentration range is rather intriguing; however, its resolution is beyond the objectives of the present study. It is noteworthly that recent experiments on flounder proximal tubule primary monolayer cultures mounted in Ussing chambers also showed a similar phenomenon. Both unidirectional reabsorptive and secretory fluxes of taurine across fish renal tubules showed saturation at lower taurine concentrations (<0.5 mM) then switched to much higher transport rates at taurine concentrations above 0.5 mM (Benyajati and Renfro, unpublished results), which is similar to the observed kinetics of BBMV taurine transport in snake kidney. Even though one was the characteristics of trans-brush-border membrane flux and the other those of trans-epithelial fluxes, the striking similarity of the effects of taurine concentrations on taurine transport seen in the kidneys of the two species that can secrete taurine suggests that this phenomenon is probably not artifactual.

The number of saturable transport systems for taurine ranging from one to three has also been reported in other cell types [12, 13]. In most cases, the discrepancies in the number of kinetic components of transport observed and the values for kinetic constants arise from the differences in the range of the substrate concentrations examined and in the types of kinetic analytical methods employed [13]. From Table 1, it can be seen that in the vertebrate kidneys, if taurine transport was studied over a narrow range of taurine concentrations, only one component of transport was usually observed. If a wider range of taurine concentrations was used, encompassing lower concentrations of taurine (<30 μM), two component systems were found. The finding of two saturable transport systems for taurine are more common for the renal basolateral membrane, probably because of these factors. Despite the many problems associated with the various renal preparations and the methods used for the determination of kinetic constants of renal taurine transport, a general pattern for the difference between the luminal and basolateral taurine transport systems could be discerned from inspection of Table 1. The transport systems for taurine on the luminal membrane generally appear to be of higher affinity (lower K_m values) than the basolateral taurine transport systems.

Earlier in vivo data on the renal handling of taurine in ophidian reptiles [2] suggest the possibility that taurine could be secreted at separate site from its reabsorptive location along the nephron of these animals. It is therefore possible that the presence of two transport system for taurine could be due to heterogeneous vesicle populations arising from brush-border membranes with differing taurine transport properties. Our present studies do not allow us to distinguish this possibility. The normal plasma concentration of taurine in the garter snake is approximately 1 μM, well below the apparent K_m (70 μM) of the high-affinity brush-border taurine transport system, suggesting that this system is probably responsible for taurine reabsorption under in vivo physiological conditions. However, under conditions such as winter hibernation, taurine concentrations in the plasma of these snakes usually increase. In cold-acclimated garter snakes, the concentration of taurine in plasma ranged from 0.5 to 1.8 mM [1]. This sugests that the low-affinity, high-capacity, Na^+-dependent taurine transport system (K_m = 1.1 mM) identified in the present study could also operate physiologically. Cold-acclimated garter snakes excreted taurine at a significantly higher rate than did control animals [1]. This could be the result of either enhanced taurine secretion or reduced taurine reabsorption, since the snake is capable of both types of renal transport. In marine fishes, net tubular secretion of taurine increased when plasma taurine concentrations were elevated [22]. It is possible that the low-affinity, high-capacity transport system for taurine in snake kidney may be involved in the process of taurine secretion in this species. A recent observation of taurine transport in mammalian kidney supports this possibility. Using in vivo and in situ microperfusion techniques, Silbernagl [24] reported that in the rat proximal tubule, taurine reabsorption and secretion occurred simultaneously. Under normal conditions, reabsorption by a low-capacity system predominates; at high plasma taurine levels the

Table 1. Estimated kinetic constants (K_m) of taurine transport in vertebrate kidneys

Species and type of preparation	Membrane location of transport system	Taurine concentration range examined (mM)	K_m[a] (mM)	Estimation method for kinetic constant	Reference
Dogfish kidney slices, 2 h uptake	Peritubular	1.0–10	9.0	Lineweaver-Burk	22
Flounder teased renal tubules, 1 h uptake	Peritubular	0.1–15	1.8	Lineweaver-Burk	16
Killifish teased renal tubules, 1 h uptake	Peritubular	0.5–3.0	5.7	Lineweaver-Burk	28
Mouse renal cortical slices, 2 h uptake	Peritubular	0.025–50	a) 0.2–0.7 b) 14–17	Eadie-Augustinson and Lineweaver-Burk	5
Rat renal cortical slices, 1–2 h uptake	Peritubular	0.001–60	a) 0.1–0.3 b) 14–15	Eadie-Augustinson and Lineweaver-Burk	6
Rat isolated renal cortical tubule segments, 10 min uptake	Peritubular	0.006–20	a) 0.12 b) 5.3	Eadie-Hofstee	11
Mouse renal basolateral membrane vesicles, 15 s uptake	Peritubular	0.1–2.0	0.36 ± 0.01(SE)	Eadie-Hofstee	19
Rat renal proximal tubules, in vivo and in situ microperfusion	Mostly luminal	0.1–2.17	Max 0.54	Estimated from apparent permeability coefficient	10
Rat renal brush-border membrane vesicles, 30 s uptake	Luminal	0.03–0.4	0.017 ± 0.002(SE)	Lineweaver-Burk	21
Rat renal brush-border membrane vesicles 30 s uptake	Luminal	0.01–0.25 0.01–10	0.04 2 K_m values (no values given)	Eadie-Hofstee	7
Rat renal brush-border membrane vesicles, 15 s uptake	Luminal	0.01–0.25	0.024 ± 0.004(SE)	Lineweaver-Burk	30
Rabbit renal brush-border membrane vesicles, 20 s uptake	Luminal (pars convoluta)	0.01–10.0	a) 0.086 ± 0.009(SE) b) 5.41 ± 0.48(SE)	Non-linear regression	14
	Luminal (pars recta)	0.01–10.0	a) 0.012 ± 0.005(SE) b) 5.62 ± 0.55(SE)		
Garter snake renal brush-border membrane vesicles, 5 s uptake	Luminal	0.001–5.0	a) 0.071 ± 0.046(SD) b) 1.11 ± 0.63(SD)	Non-linear regression	Present study

[a] a), b), indicate that two transport systems were suggested by the transport kinetics

reabsorptive mechanism is saturated and secretion by a high-capacity system takes over.

The brush-border transport of taurine by the high-affinity system in mammalian kidneys involves a stoichiometric coupling of two or more Na^+ ions. Based on the activation model, the present study suggests that two or three Na^+ ions are involved with taurine movement across snake luminal membrane. Snake renal BBMV taurine uptake is also stimulated by a Cl^- gradient [4], however the stoichiometry for the interaction between taurine and the anion Cl^- has not been determined for the snake brush-border membranes. Nevertheless, the earlier finding that brush-border taurine transport involves a net transfer of positive charge across the membrane [4] would suggest a minimal stoichiometry of the taurine carrier complex as 2 Na^+ : 1 Cl^- : 1 taurine, since taurine exists as a zwitterion at physiological pH. Therefore, the stoichiometry for the transport system of taurine in the renal brush-border membrane of the animals that can secrete taurine (i.e. snakes) appears to be similar to that observed in mammalian kidneys that reabsorb the amino acid [27, 30]. The involvement of multiple Na^+

ions as well as Cl^- in the transport process of taurine and the positive electrogenic nature of the transport potentially impart a thermodynamic advantage for uphill movement, suggesting the presence of a highly efficient transport system for taurine reabsorption on the luminal membrane of the snake renal cells. Indeed, the observed magnitude of the taurine chemical gradient normally sustained across the snake renal brush-border membranes of about 3×10^4 to 1 strongly supports this conclusion (extracellular taurine ≈ 1 μM and intracellular taurine in snake kidney cells $= 26.0 \pm 3.8$ mM; Benyajati, unpublished results). In addition, the normal plasma concentration of taurine in the garter snakes (1 μM) is well below the apparent K_m of the brush-border high-affinity, low-capacity taurine transport system (70 μM), further suggesting that taurine should be avidly reabsorbed in these animals under in vivo conditions. Since taurine is also secreted under physiological conditions in vivo in snakes, further studies are needed to resolve the nature of the mechanism by which taurine is secreted by snake renal cells. A recent study on taurine secretion in cultured flounder renal epithelium suggests that taurine efflux from cell to lumen across the luminal membrane is facilitated by a carrier-mediated transport that is not the Na^+-taurine cotransporter; the flounder taurine secretory transport system is sensitive to bromcresol green and probenecid but does not transport β-alanine [20]. Whether taurine is secreted into the snake renal tubule lumen by the low-affinity, high-capacity Na^+-dependent taurine transport system identified in the present study or by a totally different pathway remains to be elucidated. Furthermore, in renal epithelium the direction of net transepithelial movement of taurine is also critically determined by the influx and efflux transport processes across the basolateral membranes of the cells. The process of renal tubular reabsorption or secretion of taurine will be better understood when the transport events at both luminal and basolateral membranes of the renal cells can be simultaneously described.

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