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Taurine Transport by Rabbit Kidney Brush-Border Membranes: Coupling to Sodium, Chloride, and the Membrane Potential

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Summary. Ion dependence and electrogenicity of taurine uptake were studied in rabbit renal outer cortical brush-border membrane vesicles isolated by differential precipitation. Na^+-D-glucose cotransport was followed in parallel to monitor changes in the membrane potential. Concentrative taurine flux was dependent on a chemical and/or an electrical Na^+ gradient (K^+ diffusion potential) and could be completely inhibited by other β-amino acids. It displayed a specific anion requirement ($Cl^- \geq Br^- \gg SCN^- > I^- > NO_3^-$). At chemical Na^+ equilibrium, Cl^- gradients, depending on their orientation, stimulated or inhibited taurine uptake more than could be attributed solely to electrical anion effects, although a Cl^- gradient alone could not energize an overshoot. Furthermore, taurine tracer exchange was significantly stimulated by Cl^- as well as Br^-. The Cl^- stoichiometry was found to be one, whereas taurine transport, in the presence of Cl^-, was sigmoidally related to the Na^+ concentration, resulting in a coupling ratio of 2 to 3 Na^+ : 1 taurine. Upon Cl^- replacement with gluconate, taurine uptake showed a reduced potential sensitivity and was no longer detectably affected by the Na^+ concentration (up to 150 mM). These results suggest a 2 to 3 Na^+ : 1 Cl^- : 1 taurine cotransport mechanism driven mainly by the Na^+ gradient, which is sensitive to the membrane potential due to a negatively charged empty carrier. Cl^- appears to stimulate taurine flux primarily by facilitating the formation of the translocated solute-carrier complex.

Key Words taurine · transport · kidney · sodium · chloride · electrogenicity

Introduction

Taurine (Tau), the naturally occurring sulfonic acid analog of β-alanine, is found in a variety of mammalian cells in relatively high concentrations and is known to be synthesized primarily in brain, heart, liver, and kidney (Jacobsen & Smith, 1968). Despite its wide distribution, the function of taurine in terrestrial animals is still unclear, except for its importance in bile acid secretion by the liver, and neurotransmission in the mammalian central nervous system (Curtis & Johnston, 1974). A role in cell volume regulation, known to be a major function of taurine in many marine vertebrate and invertebrate species (Gilles, 1974; King & Goldstein, 1983), has so far only been suggested for Ehrlich ascites tumor cells (Hoffmann & Hendril, 1976). In contrast, the renal handling of taurine has been relatively well investigated. Almost 90% of the filtered load are normally reabsorbed in the proximal tubule (Eisenbach, Weise & Stolte, 1975) via a β-amino acid-specific Na^+-cotransport mechanism in the brush-border membrane (Goldman & Scriver, 1967; Hammerman & Sacktor, 1978; Rozen, Tenenhouse & Scriver, 1979; Chesney, Gusowski & Friedman, 1983). Na^+-dependent taurine uptake by rat renal brush-border membrane vesicles (BBMV), as well as β-alanine translocation in BBMV isolated from dog kidney, have been found also to specifically require Cl^- (Chesney et al., 1985; Turner, 1986). Such a Cl^- dependence is characteristic of many Na^+-coupled transport systems for amino acids (Kanner, 1978; Ellory, Jones & Young, 1981; Mayor et al., 1981; Corcelli & Storelli, 1983; Corcelli, Scalera & Storelli, 1985) and for structurally related substances, such as noradrenalin (Sanchez-Armass & Orrego, 1977) and serotonin (Nelson & Rudnick, 1982), in various tissues. The mode of Cl^- action in renal and intestinal amino-acid reabsorption is, however, still not well understood. Chesney et al. (1985) observed an inhibitory effect of intravesicular Cl^- on taurine uptake by rat renal BBMV, and therefore suggested that the anion acts on the external side of the membrane, probably by inducing a conformational change of the carrier. On the other hand, Turner (1986) recently reported that in brush-border vesicles from dog proximal tubule, concentrative β-alanine translocation can be driven by a Cl^- gradient alone, indicating Cl^- cotransport. The purpose of the present study on BBMV isolated

from rabbit kidney cortex by differential precipitation was to further elucidate the role of Cl^- in renal β-amino acid transport. In particular, the question of energetic (flux coupling) versus solely catalytic activation by the anion was investigated. The results presented below suggest that chloride is actually translocated across the membrane together with taurine and 2 to 3 sodium ions, and that the process is electrogenic, i.e. stimulated by a negative potential on the *trans* side of the membrane.

Materials and Methods

Brush-Border Membrane Preparation

Renal brush-border vesicles were isolated by a modified magnesium precipitation method originally described by Booth and Kenny (1974). Adult male New Zealand White rabbits, weighing 2.5 to 3.0 kg, were killed by a blow to the head and exsanguinated; the kidneys were rapidly excised and placed in ice-cold ST-buffer (250 mM sucrose, 10 mM triethanolamine-H_2SO_4, pH 7.4). The superficial cortical tissue was carefully dissected and frozen at −70°C for at least 24 hr prior to preparing the membranes. For each preparation, 30 g of the cortical tissue were quickly thawed at 37°C in homogenization buffer (10 mM mannitol, 2 mM Tris-HCl, pH 7.1), minced with scissors and homogenized for 30 sec at full speed in a precooled Waring blender. Three Mg^{2+} precipitation steps followed. The final brush-border membrane pellet was resuspended in vesicle buffer (200 mM mannitol, 20 mM HEPES-Tris, pH 7.4) by gentle suction first through a 23-gauge, then through a 26-gauge syringe needle. The protein concentration was adjusted to 10 mg/ml. All procedures were carried out at 0 to 4°C. The membranes were stored at −70°C until use.

Uptake Measurements

Uptake of ^{3}H-taurine or ^{3}H-D-glucose, was followed at 25°C using a rapid filtration technique (Hopfer et al., 1973). The reaction was initiated by adding 20 μl of the vesicle suspension to 130 μl incubation medium, containing 0.01 mM taurine and 6.8 μCi ^{3}H-taurine, or 0.1 mM D-glucose and 6.2 μCi ^{3}H-D-glucose. The detailed composition of the incubation media is given in the Figure and Table legends. At the time points indicated, uptake was terminated by diluting 20 μl of the reaction mixture into 1 ml of ice-cold stop solution (for taurine transport studies: 200 mM mannitol, 300 mM NaCl, and 20 mM HEPES-Tris, pH 7.4; for glucose uptake: 100 mM mannitol, 150 mM NaCl, 20 mM HEPES-Tris, pH 7.4, and 0.5 mM phlorizin). The vesicles were separated from the medium by filtration onto a Millipore filter (HA 0.45 μm), and washed once with 3 ml of stop solution. The filters with the retained membranes were dissolved in HP/b Ready Solv (Beckmann Instruments, Muenchen, FRG) and assayed for radioactivity by liquid scintillation counting. All uptake experiments were carried out in duplicate. In order to avoid bacterial contamination, incubation solutions and vesicle buffer were filtered prior to use (Millex-GS, 0.22 μm). The 15-sec time point was found to lie well within the linear phase of taurine uptake and was, therefore, considered to represent initial uptake.

Determination of Protein Content and Enzyme Activities

The brush-border vesicles, as well as the cortex homogenate and the pooled discarded pellets and supernatants, were routinely assayed for leucine aminopeptidase (LAP; EC 3.4.11.1) as marker enzyme for brush-border membranes and Na^+, K^+-ATPase (EC 3.6.1.3) for basolateral membranes immediately after the preparation. LAP activity was determined in a 100 mM mannitol-20 mM HEPES-Tris buffer, pH 7.4, with 1.0 mM leucine-*p*-nitroanilide as substrate (37°C, 380 nm). The specific activity of the vesicle preparation averaged 70.3 ± 17.2 μmol substrate hydrolyzed/mg protein · hr (mean ± SD, n = 21) and was enriched 11.6-fold compared to the cortex homogenate. Na^+, K^+-ATPase activity was assayed according to the method of Kinne, Schmitz and Kinne-Saffran (1971) and averaged 3.4 ± 1.1 μmol substrate hydrolyzed/mg protein · hr (n = 17). The mean ratio of brush-border membrane/homogenate activity was 0.43, indicating little basolateral contamination of the vesicle suspension. Total recovery of enzyme activity was 100.0 ± 7.4% and 78.8 ± 7.1% (means ± SD, n = 16) for LAP and Na^+, K^+-ATPase, respectively. Proteins were measured after precipitation with cold 10% trichloroacetic acid essentially as described by Lowry et al. (1951), using bovine serum albumin as a standard.

Statistical Analysis

Data are expressed as the means ± standard deviation ($\bar{x}$ ± SD) of the number of experiments indicated by n, representing an equal number of different membrane preparations. Significant differences in uptake between two means as calculated using the Student's t-test for group data are indicated by P values.

Chemicals

[2-^{3}H(N)]-Taurine (17.6 Ci/mmol) and [6-^{3}H(N)]-D-glucose (33.1 Ci/mmol) were purchased from New England Nuclear (Dreieich, FRG). All other chemicals were obtained in the highest purity grade.

Results

General Characteristics of Taurine Uptake

Previous studies on rabbit, rat, and dog renal brush-border membrane vesicles have shown that the common transport mechanism for taurine and other β-amino acids is energized by the Na^+ gradient (Hammerman & Sacktor, 1978; Rozen et al., 1979; Rozen & Scriver, 1982; Turner, 1986), but also specifically requires the presence of chloride (Chesney et al., 1985; Turner, 1986). The vesicle preparation used in the present study was first tested for these properties in order to confirm its suitability for investigating the energetics and coupling to inorganic ion fluxes of taurine transport across the renal brush-border membrane. At an initial 100 mM NaCl gradient, taurine uptake at 12.6 μM taurine dis-

played an 'overshoot' of 2 to 3 times the equilibrium concentration after approximately 3 min of incubation. Replacement of Na^+ with K^+ resulted in an 80% decrease of the initial uptake rate and completely prevented intravesicular taurine accumulation. Under salt equilibrium conditions (NaCl or KCl) again no overshoot was observed, but uptake was significantly stimulated by Na^+ over K^+, which is also consistent with a Na^+-cotransport mechanism.

The Na^+-dependent component of ^3H-taurine flux could be completely inhibited by 5 mM nonlabeled taurine ('tracer replacement'), β-alanine or hypotaurine, not, however, by the α-amino acid glycine, whereas uptake in KCl-media was unaffected by any of the amino acids tested.

Cl^- replacement under conditions of a Na^+-salt gradient (Table 1) revealed a specific anion requirement for taurine transport with a similar efficiency pattern ($Cl^- \geq Br^- \gg SCN^- > I^- > NO_3^-$) as that recently reported for β-alanine uptake into dog renal outer cortical BBMV (Turner, 1986). Except for Br^-, all ions other than Cl^- resulted in a strongly decreased initial uptake rate and lowered or entirely prevented the overshoot. These effects could not be due solely to different anion diffusion potentials, since the sequence does not correspond to the passive ion permeability of the membrane as reflected by changes in D-glucose transport—known to be Cl^- independent (Ullrich, 1979)—under identical conditions (Table 1).

Table 1. Anion specificity of taurine uptake[a]

Anion	*n*	Solute uptake relative to that in Cl^- medium	
		(*A*) Taurine	(*B*) D-glucose
Cl^-	(4)	1.00 ± 0.25	1.00 ± 0.15
Br^-	(3)	1.00 ± 0.17	1.31 ± 0.10
I^-	(3)	0.59 ± 0.10	1.62 ± 0.17
F^-	(3)	0.14 ± 0.01	0.71 ± 0.14
SCN^-	(4)	0.76 ± 0.11	1.67 ± 0.19
NO_3^-	(4)	0.48 ± 0.06	1.38 ± 0.19
Gluconate$^-$	(4)	0.16 ± 0.04	0.54 ± 0.09

[a] The incubation media contained 20 mM HEPES-Tris, pH 7.4, 12.6 μM [^3H]-taurine or 106 μM [^3H]-D-glucose and 100 mM of one of the anions listed above, as Na^+ salt. Solute uptakes at 15 sec were expressed as % equilibrium value and then normalized to the uptake in Cl^- medium set to one. They are means ± SD of the number of experiments indicated by *n*.

Taurine Transport Electrogenicity at Different Ionic Conditions

In contrast to Turner (1986), the membrane potential in rabbit renal BBMV could, under certain conditions, neither be satisfactorily clamped to a K^+ diffusion potential generated by an initial chemical K^+ gradient (150 mM inside/20 mM outside) in the presence of 12 μg valinomycin/mg protein, nor completely short-circuited by a 150-mM K^+ equilibrium concentration (+ valinomycin). For example, at a 100-mM anion gradient with $[Na^+]_{inside} = [Na^+]_{outside}$, D-glucose uptake at 15 sec differed by more than 40% between a nitrate and a gluconate medium, both in the presence of a K^+ diffusion potential (362 *vs.* 197 pmol/mg protein, $n = 3$) and at identical K^+ inside and outside the vesicles (77.3 *vs.* 40.0 pmol/mg protein, $n = 3$). Therefore, since β-amino acid (β-alanine) translocation in the same experimental system has previously been shown to be an electrogenic, i.e. charge-translocating process (Hammerman & Sacktor, 1978), we studied the potential sensitivity of taurine flux in comparison with that of Na^+-D-glucose transport under various conditions, to examine the suitability of the latter as a monitoring system for unspecific electrical anion effects. Furthermore, it was of interest, whether taurine transport electrogenicity is affected by anion replacement. As shown in Fig. 1, at a 100-mM

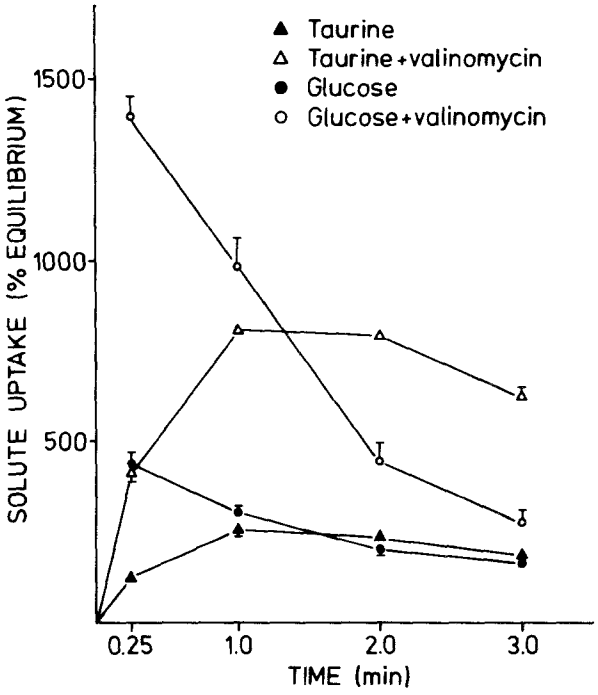

Fig. 1. Potential sensitivity of taurine and D-glucose transport. The membranes were preincubated at 25°C, first with valinomycin (12 μg/mg protein) (△, ○) or an aliquot of pure ethanol (▲, ●) for 5 min, and then for 1 hr in a medium containing 100 mM K^+-gluconate, 50 mM KCl, 200 mM mannitol, and 20 mM HEPES-Tris, pH 7.4. The incubation media contained 50 mM Na^+-gluconate, 50 mM NaCl, 300 mM mannitol, 20 mM HEPES-Tris, pH 7.4, and 12.6 μM [^3H]-taurine (△, ▲) or 106 μM [^3H]-D-glucose (○, ●). Values are means ± SD ($n = 3$)

Table 2. Effect of anion replacement on taurine and D-glucose transport electrogenicity[a]

		Potential sensitivity of solute uptake at 15 sec $\frac{J_2 - J_1}{J_1}$		
		Cl^-	NO_3^-	Gluconate$^-$
(*a*) 100 mM Na^+-A^- gradient:	taurine	3.39 ± 0.74	2.26 ± 0.32	1.59 ± 0.28
	glucose	2.79 ± 0.35	2.17 ± 0.31	2.78 ± 0.26
		NS	NS	$P < 0.01$
(*b*) 100 mM Na^+ gradient:	taurine	2.25 ± 0.24	1.02 ± 0.07	1.35 ± 0.13
$[A^-]_i = [A^-]_o$ (50 mM)	glucose	2.20 ± 0.22	1.64 ± 0.21	3.01 ± 0.46
		NS	$P < 0.01$	$P < 0.01$
(*c*) 100 mM A^- gradient:	taurine	4.94 ± 0.64	1.92 ± 0.12	1.42 ± 0.03
$[Na^+]_i = [Na^+]_o$ (50 mM)	glucose	5.44 ± 0.24	4.25 ± 0.41	4.18 ± 0.38
		NS	$P < 0.001$	$P < 0.001$

[a] All media were buffered with 20 mM HEPES-Tris, pH 7.4. The incubation media contained 12.6 μM [^{3}H]-taurine or 106 μM [^{3}H]-D-glucose. Cation or anion concentration differences between preincubation and incubation medium were balanced with N-methyl-D-glucamine or gluconate, respectively. The osmolarity of the two corresponding media was adjusted with mannitol. Under all three conditions vesicles were preloaded with 150 mM K^+ for 1 hr at 25°C. Membranes for (*a*) and (*c*) were preincubated with valinomycin (12 μg/mg protein) for 5 min at 25°C prior to K^+-preloading; the incubation media contained 150 mM K^+ or were K^+ free. Membranes for (*b*) were preincubated with valinomycin or an aliquot of pure ethanol (5 min, 25°C) prior to K^+ preloading; the incubation medium was K^+ free. The concentrations of Na^+ and Cl^-, NO_3^-, or gluconate were as indicated. The potential sensitivity was calculated using the equation $J_2 - J_1/J_1$, where J_2 and J_1 represent the flux in the presence or absence of a K^+ diffusion potential, respectively (for a detailed discussion *see* Heinz et al., 1988). Values are means ± SD ($n = 3$). Significant differences in electrosensitivity between taurine and D-glucose uptake are indicated by P values. NS = not significant; A^- = anion; i = inside; o = outside.

NaCl gradient taurine uptake into vesicles preloaded with 150 mM K^+ could be significantly stimulated by the addition of valinomycin (12 μg/mg protein). In the presence of the ionophore, generating a transmembranal electrical potential difference (intravesicular negative), the initial transport rate was increased to more than threefold and a higher transient substrate accumulation occurred, effects that were almost identical to those on D-glucose uptake (Fig. 1). The same appears to hold for conditions of a 100-mM Na^+ or Cl^- gradient alone, with the counter-ion (Cl^- or Na^+, respectively) at a lower concentration (50 mM) equal on both sides of the membrane (Table 2). However, the potential sensitivity of taurine uptake compared to that of D-glucose transport seems to decrease upon Cl^--replacement with NO_3^- or gluconate (Table 2). Corrections for unspecific electrical effects on taurine transport based on changes in D-glucose flux are apparently justified only for media containing both Na^+ and Cl^- and thus do not allow for an exact determination of the anion specificity sequence (*see above*).

Affinity and Stoichiometry for Chloride

The affinity of the Na^+-taurine cotransport mechanism for chloride was determined according to the 'activation method' (Turner, 1983), i.e. by measuring initial uptake at different extravesicular Cl^- concentrations. The measured flux rates were corrected for electrical Cl^- effects according to the changes observed in D-glucose uptake, which has previously been found to be a linear function of the membrane potential in rabbit renal brush-border membrane vesicles (Beck & Sacktor, 1978). As shown in Fig. 2, Cl^--dependent taurine transport was a hyperbolic function of the extravesicular Cl^- concentration, indicating a 1 Cl^- : 1 Tau stoichiometry. Different ways of graphical analysis (e.g. Eadie-Hofstee plot: Fig. 2 insert) substantiated Michaelis-Menten-type kinetics and revealed an apparent $K_{0.5(Cl^-)}$ of approximately 110 mM.

Mode of Cl^- Action—Cotransport or Catalytic Activation

In order to investigate whether Cl^- acts as an additional driving force for concentrative taurine transport or only activates the carrier without being translocated, we studied—under conditions of chemical Na^+ equilibrium—the effect of Cl^- gradients on taurine uptake relative to transport at an identical Cl^- concentration present on both sides of the membrane. As Fig. 3 shows, Cl^- concentration

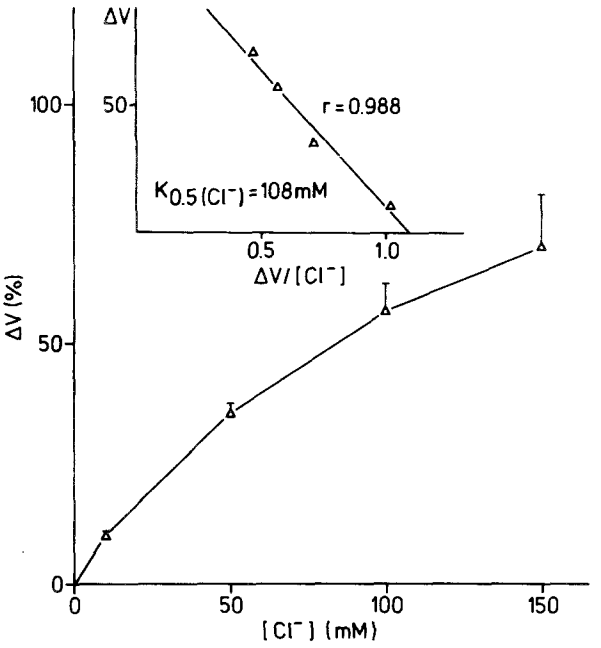

Fig. 2. Chloride concentration dependence of taurine transport. The membranes were preincubated at 25°C, first with valinomycin (12 μg/mg protein) for 5 min, then for 1 hr in the following medium: 150 mM K^+-gluconate, 50 mM Na^+-gluconate, and 20 mM HEPES-Tris, pH 7.4. The incubation media contained 20 mM HEPES-Tris, pH 7.4, 12.6 μM [^{3}H]-taurine, 50 mM Na^+, and Cl^- at the concentration indicated. The cation concentration was balanced with N-methyl-D-glucamine, the anion concentration with gluconate. The osmolarity was adjusted to that of the preincubation medium with mannitol. Initial uptake was measured at 15 sec and expressed as % equilibrium. Cl^--dependent flux (ΔV) was then calculated by subtracting the uptake in Cl^--free medium from the total taurine taken up at each Cl^- concentration and then dividing by the potential effect of the anion gradient as obtained from D-glucose transport followed under identical conditions (uptake at X mM Cl^-/uptake at 0 mM Cl^-). Values are means ± SD (n = 3). An Eadie-Hofstee plot of the data is shown in the insert

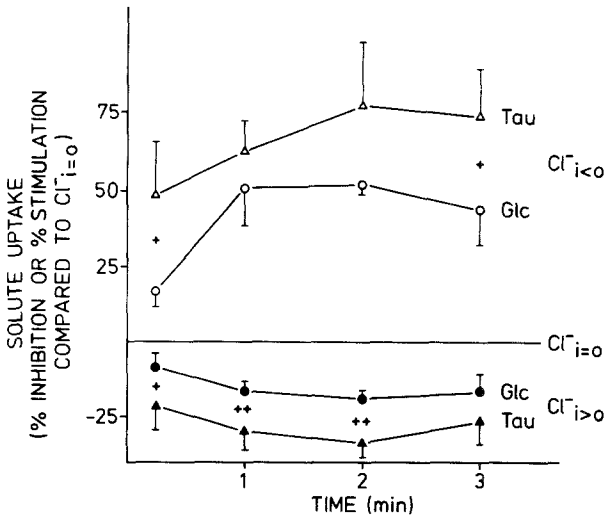

Fig. 3. Effect of differently oriented Cl^- gradients on taurine and D-glucose transport at Na^+ equilibrium. The membranes were pretreated with valinomycin (12 μg/mg protein; 5 min at 25°C), and then preincubated for 1 hr at 25°C in one of the following media: (i) For $[Cl^-]_{i=o}$ and $[Cl^-]_{i>o}$: 50 mM K^+-gluconate, 100 mM KCl, 50 mM Na^+-gluconate, and 20 mM HEPES-Tris, pH 7.4. (ii) For $[Cl^-]_{i<o}$: 150 mM K^+-gluconate, 50 mM Na^+-gluconate, and 20 mM HEPES-Tris, pH 7.4. The incubation media contained 50 mM Na^+-gluconate, 20 mM HEPES-Tris, pH 7.4, 12.6 μM [^{3}H]-taurine (△, ▲) or 106 μM [^{3}H]-D-glucose (○, ●), and: (I) For $[Cl^-]_{i=o}$ and $[Cl^-]_{i<o}$: 50 mM N-methyl-D-glucamine-gluconate and 100 mM N-methyl-D-glucamine-Cl^-, or (II) for $[Cl^-]_{i>o}$: 150 mM N-methyl-glucamine-gluconate. Values are means ± SD (n = 4) and expressed as % stimulation (△, ○) or % inhibition (▲, ●) as compared to uptake at $[Cl^-]_{i=o}$. Significant differences between taurine and D-glucose uptake as calculated using the Student's t-test for group data are indicated: + ($P < 0.05$) and ++ ($P < 0.01$). Tau = taurine; Glc = D-glucose

differences, depending on their orientation, stimulated ($Cl_o > Cl_i$) or inhibited ($Cl_o < Cl_i$) the uptake more than could be accounted for solely by electrical effects (D-glucose transport!). These results indicate that taurine translocation is energetically activated by Cl^-; i.e. that Cl^- is cotransported, even though a Cl^- gradient alone was not sufficient to energize an overshoot (*not shown*).

This hypothesis was further tested by assaying for transstimulation of taurine tracer exchange (vesicles preloaded with nonlabeled taurine) by Cl^- or Br^- compared with NO_3^-, at an identical external Cl^- concentration. Replacement of intravesicular NO_3^- with Cl^- almost doubled the initial rate (15 sec) of ^{3}H-taurine influx (Table 3). A statistically significant but lower transstimulation (50%) could also be induced by Br^-. These findings can be best explained by Cl^--taurine cotransport, since transstimulation is regarded as a good indication for carrier-mediated translocation of the stimulating solute (Murer & Kinne, 1980). It should be noted that, although initially no Na^+ was present inside the vesicles, it enters much faster than taurine and is thus available for exchange. Unexpectedly, also Na^+-independent tracer exchange was slightly enhanced by intravesicular Cl^-. This cannot be due to an ability of K^+ to partly substitute for Na^+, since taurine uptake in KCl media did not display saturability or substrate specificity, but might reflect initial changes in vesicular volume.

Sodium Stoichiometry and Affinity

The comparatively small potential-independent effect of Cl^- gradients on Na^+-taurine uptake, as well as its electrogenicity, suggested a higher stoichiometry of the transport system for Na^+ than for Cl^-. Therefore, the dependence of the initial transport rate (15 sec) on the extravesicular Na^+ concentra-

Table 3. Transstimulation of taurine uptake by anions (tracer exchange experiments)[a]

	A^-	Taurine uptake (% equilibrium)			
		10 sec	15 sec	45 sec	60 sec
1. Na^+:	(*a*) NO_3^-	25.7 ± 2.3	33.0 ± 2.2	79.8 ± 8.0	90.5 ± 8.1
	(*b*) Br^-	39.6 ± 3.1	49.9 ± 5.0	97.7 ± 10.1	107.9 ± 10.9
		$P < 0.01$	$P < 0.01$	NS	NS
	(*c*) Cl^-	49.1 ± 3.6	60.1 ± 6.2	107.1 ± 12.2	115.2 ± 14.9
		$P < 0.001$	$P < 0.01$	$P < 0.05$	NS
2. K^+:	(*a*) NO_3^-	16.2 ± 1.9	20.2 ± 1.0	39.1 ± 3.0	42.8 ± 1.7
	(*c*) Cl^-	24.7 ± 2.5	27.7 ± 1.3	44.5 ± 6.7	45.5 ± 2.9
		$P < 0.01$	$P < 0.01$	NS	NS

[a] The vesicles were preincubated for 1 hr at 25°C in one of the following media: 200 mM mannitol, 20 mM HEPES-Tris, pH 7.4, 12.6 μM taurine and (*a*) 50 mM KNO_3, (*b*) 50 mM KBr, or (*c*) 50 mM KCl. The incubation media contained: 1. 100 mM Na^+-gluconate, or 2. 100 mM K^+-gluconate, 20 mM HEPES-Tris, pH 7.4, 12.6 μM [^{3}H]-taurine and 45 mM K^+ salts (NO_3^- or Cl^-). The final extravesicular anion concentrations at the beginning of the incubation were as follows: (*a*) 10 mM Cl^-, 40 mM NO_3^-; (*b*) 10 mM Cl^-, 35 mM NO_3^-, 5 mM Br^-; (*c*) 10 mM Cl^-, 40 mM NO_3^-. Values are means ± SD (n = 3). Significant differences to the respective NO_3^- controls (*a*) as obtained using the t-test for group data are indicated by P values. NS = not significant, A^- = anion.

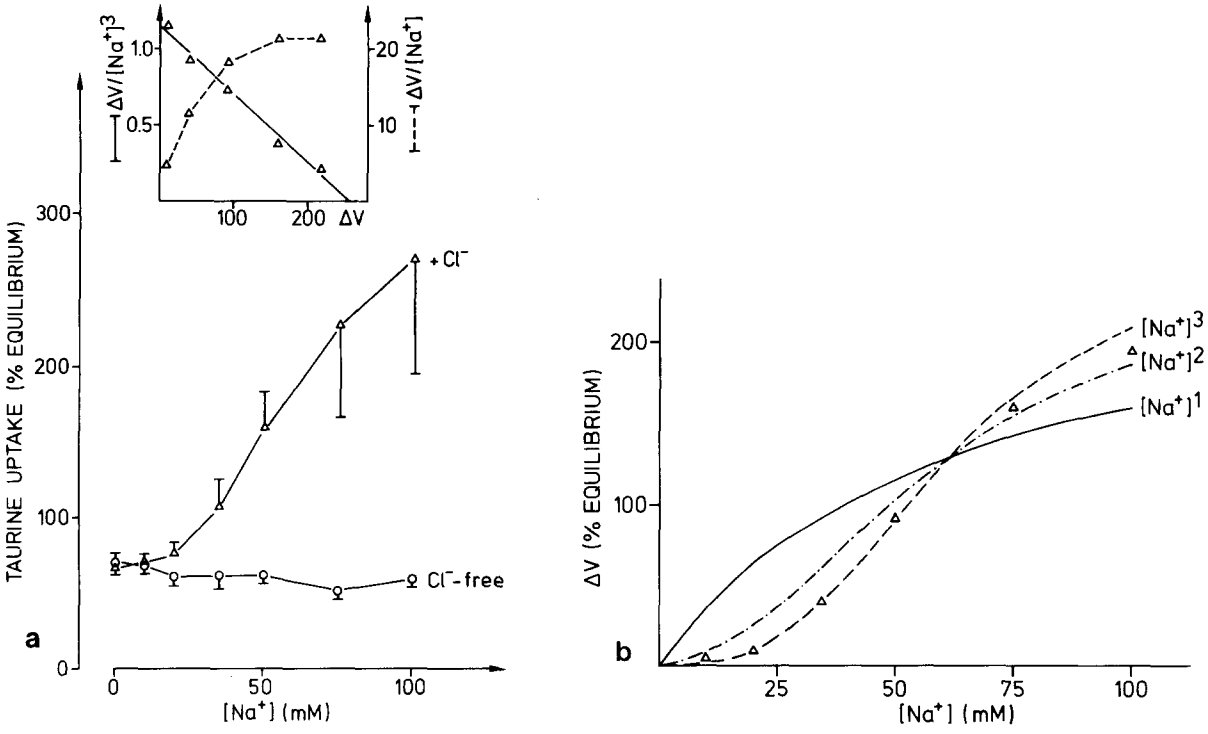

Fig. 4. Sodium concentration dependence of taurine uptake in the presence and absence of chloride. The membranes were first preincubated with valinomycin (12 μg/mg protein; 5 min at 25°C) and then for 1 hr at 25°C in media containing 100 mM K^+-gluconate, 200 mM mannitol, 20 mM HEPES-Tris, pH 7.4, and 50 mM KCl (△) or 50 mM K^+-gluconate (○). The incubation media contained 20 mM HEPES-Tris, pH 7.4, 12.6 μM [^{3}H]-taurine, 50 mM Cl^- (△) or 50 mM gluconate (○), and Na^+ in the concentration indicated. Cation and anion concentration were balanced with N-methyl-D-glucamine and gluconate, respectively. The osmolarity was adjusted to that of the preincubation medium with mannitol. Initial uptake (15 sec) is expressed as % equilibrium. Values are means ± SD (n = 5 (△) or n = 3 (○)). (*a*) Total flux (V) is plotted as a function of the Na^+ concentration. In the insert, Eadie-Hofstee plots of the Na^+-dependent taurine uptake (ΔV) with $[Na^+]^1$ and $[Na^+]^3$ are shown. As unit for the Na^+ concentration (10^{-2} M) was used in the calculations. (*b*) The kinetic parameters obtained by Eadie-Hofstee analysis (Fig. 4*a*) were used to calculate theoretical Na^+-dependent flux rates for different Na^+ stoichiometries according to the equation $V = V_{max} \times [Na^+]^p / K^p_{0.5} \times [Na^+]^p$. For comparison, the experimental data (△) are included in the Figure

tion was studied in the presence and in the absence of Cl^-. In order to enhance differences in uptake at low Na^+ gradients and to reduce electrical effects of increasing Na^+ concentrations, a vesicle-outward-directed K^+ diffusion potential was generated. Figure 4*a* illustrates the sigmoidal behavior of Cl^--dependent transport, which was observed in each individual experiment, indicating a coupling ratio (ν) for Na^+ larger than one. Eadie-Hofstee analysis yielded correlation coefficients for linear regression of $r = 0.305$ for $\nu = 2$, $r = 0.994$ for $\nu = 3$, and $r = 0.881$ for $\nu = 4$, suggesting a 3 Na^+ : 1 Tau stoichiometry (Fig. 4*a*, insert). Substituting the kinetic parameters derived from this plot ($K_{0.5(Na^+)} = 61$ mM, $V_{max} = 260\%$ *Eq.*) in the following Hill-type equation (Turner, 1983):

$$V = V_{max} \cdot [A^\nu]/(K^\nu_{0.5} + [A^\nu])$$

(A = activator), the theoretical Na^+ concentration dependence of taurine uptake at different coupling ratios was calculated. As shown in Fig. 4*b*, the experimental data (Δ) at low Na^+ concentrations are best described by the curve obtained for $\nu = 3$,whereas above 50 mM Na^+ the stoichiometry appears to be closer to 2.

In contrast, increasing Na^+ had no detectable effect on taurine flux in Cl^--free media, which at 150 mM Na^+ was still not significantly different from the Na^+-independent uptake (Fig. 4*a*).

Discussion

The results of the present study confirm for the system under investigation the basic characteristics of β-amino acid transport across the brush-border membrane of the mammalian renal proximal tubule, such as its substrate specificity, Na^+ gradient dependence, electrogenicity and a specific chloride requirement, thus verifying the functional integrity of the vesicles. Although the exact anion specificity sequence could not be determined (*see* "Taurine Transport Electrogenicity . . ."), Table 1 clearly shows that of all anions tested only Br^- was an effective substitute for Cl^-, in agreement with findings on other anion-dependent Na^+-amino acid transport systems (Imler & Vidaver, 1972; Corcelli & Storelli, 1983; Bogé, Roche & Pérès, 1985).

Concerning the mode of Cl^- action, strong indications are provided that the anion not only catalytically activates the taurine carrier at a modifier site but is actually cotransported: First, the stimulation or inhibition of taurine uptake by differently oriented Cl^- gradients under conditions of chemical Na^+ equilibrium could not be completely attributed to changes in the membrane potential (Fig. 3), and second, taurine tracer exchange was transstimulated by Cl^- and Br^- (Table 3). That direct evidence for the energetic force of a Cl^- potential—in that it could alone energize an overshoot of taurine uptake—could not be obtained, might be related to several factors: (i) The driving force is known to increase with the power of the coupling ratio (Aronson, 1981), and the higher Na^+ than Cl^- stoichiometry we determined therefore greatly reduces the share of Cl^- in taurine transport energetics. (ii) The renal β-amino acid transport system has a low capacity (Dantzler & Silbernagl, 1976), whereas the Cl^- permeability of rabbit renal microvillus membranes in vitro is possibly high (Seifter, Knickelbein & Aronson, 1984), so that the Cl^- gradient might have dissipated before taurine has even reached the equilibrium concentration. In a different experimental approach, starting with the substrate (in this case β-alanine) already at equilibrium, Turner (1986) could actually demonstrate in a dog kidney vesicle preparation that a Cl^- gradient can drive uphill β-amino acid translocation.

This author also determined a coupling ratio of one for Cl^- and at least two for Na^+, which agrees well with the results presented above. Figure 4 suggests that even 3 Na^+ might be cotransported with each taurine, although it has to be noted that the 'activation method' does not distinguish between energetic and catalytic activation (Turner, 1983), i.e. not all Na^+ ions need to be translocated. Although the Na^+ stoichiometry appeared to change from 3 to 2 with increasing Na^+ concentration (Fig. 4*b*), this might be due to a faster dissipation of the membrane potential (inside negative) because of higher Na^+ influx and not an actually different coupling ratio.

In the absence of Cl^- (gluconate medium), taurine uptake was not detectably affected by the Na^+ concentration. However, carrier-mediated taurine translocation appears to occur even in Cl^--free media, since uptake under these conditions was still clearly electrogenic (Table 2). Passive diffusion of the amino acid taurine, existing predominantly in the zwitterionic form at pH 7.4 ($pK_1 = 1.5$, $pK_2 = 8.7$) should not be significantly influenced by the membrane potential. Taken together, the latter two findings suggest that Cl^- replacement considerably reduces the accessibility and/or affinity of the taurine carrier for Na^+, so that no change in uptake can be observed within the Na^+ concentration range tested. (From our results it cannot be deduced, however, whether this is due to gluconate binding with a lower efficiency or not binding at all to the transport system.) Beyond that, the potential sensitivity of taurine uptake compared to that of D-glu-

cose transport appeared to also decrease upon replacement of Cl^- with gluconate (or NO_3^-; Table 2). Thus, attempts to explain the mechanism underlying the anion substitution effects need to take into consideration which step in the translocation cycle might confer electrogenicity on taurine transport, (i) a positively charged fully loaded or (ii) a negative empty carrier, as has been previously suggested for active renal D-glucose reabsorption (Aronson, 1978). Most likely, although not necessarily (for a detailed discussion *see* Heinz, Sommerfeld & Kinne, 1988), the transfer of only one net charge is involved in taurine translocation, since it was similarly responsive to changes in the membrane potential as D-glucose uptake at both 100 mM Na^+ (gradient) and 50 mM Na^+ (equilibrium) (Table 2). In case of a neutral carrier molecule, a 2 Na^+ : 1 Cl^- : 1 Tau stoichiometry would therefore be more probable, but such a model could not explain the decreased potential sensitivity in the presence of gluconate, regardless whether gluconate can actually bind or not. It could be argued that the 15-sec uptake of D-glucose does not represent the initial rate because of the high capacity of this transport system, and that the differences in the apparent electrical responsiveness of taurine translocation might therefore be due to an underestimate of the actual membrane potential in the presence of Cl^- and an overestimate in its absence. This, however, is incompatible with a comparable ratio of taurine-/D-glucose-transport potential sensitivity observed at different Na^+ concentrations (50 mM equilibrium and 100 mM gradient; Table 2). For a similar reason, those differences also cannot be explained by a higher Na^+-dependent/Na^+-independent taurine uptake ratio in Cl^- than in gluconate media. We, therefore, currently favor the view that the empty carrier is negatively charged and rate limiting in the presence of Cl^-. In gluconate media, where taurine uptake is also electrogenic, the taurine-translocating complex might have the form Na^+-Tau-C^- (C^- = carrier) or 2 Na^+-gluconate$^-$-Tau-C^-, i.e. be electroneutral. A reduced fractional rate-limitancy of the translocation of the empty carrier relative to the substrate translocating step could then explain the effect of Cl^- replacement on taurine transport potential sensitivity (*see* Heinz et al., 1988). Further experiments are, however, necessary to provide stronger evidence for a translocation of partially loaded carrier species.

In summary, we propose a 2 to 3 Na^+ : 1 Cl^- : 1 taurine cotransport mechanism driven mainly by the electrochemical Na^+ gradient, with strong ion cooperativity, that is electrogenic because of a negative carrier molecule. Such a system, which appears to represent the major route for taurine translocation across the renal brush-border membrane, would be strongly reabsorptive under in vivo conditions. The high tubular lumen/cell Na^+ and Cl^- gradients as well as the membrane potential would provide the necessary driving force for strong intracellular β-amino acid accumulation, facilitating passive efflux at the basolateral side. The affinity for Na^+ and Cl^- determined in vitro, both below the actual ion concentrations in the glomerular filtrate, are most likely underestimates. Assuming cooperative substrate interaction, the higher concentration of all three solutes in primary urine (approx. 145 mM Na^+, 125 mM Cl^-, and 30 μM taurine; (Jacobsen & Smith, 1968; Biagi & Giebisch, 1979) than in the corresponding experiments (Figs. 2 and 4) would result in even more favorable conditions in vivo.

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