Taurine Transport in Renal Brush-Border-Membrane Vesicles

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Taurine transport in isolated brush-border-membrane vesicles from rat kidney is concentrative and it is driven by the Na^+ gradient and transmembrane potential difference; binding is not a significant component of net uptake. The Na^+ -dependent component of net uptake is saturable with an apparent K_m of $17\ \mu\text{M}$. The taurine-transport mechanism is selective for β -amino compounds.

Taurine (2-aminoethanesulphonic acid), a β -amino compound, is present in mammalian body fluids (blood plasma, breast milk and urine) and tissues (myocardium, platelets, retina and nerve tissue) (Holden, 1962). Although its function is not well understood, it is involved with excitatory activity in the nervous system (Barbeau *et al.*, 1976) and myocardium (Huxtable & Chubb, 1977).

Taurine is the most abundant ninhydrin-positive substance in rodent urine (Holden, 1962) and its renal reabsorption is achieved by a β -amino acid-transport system (Chesney *et al.*, 1976; Dantzer & Silbernagl, 1976). Strain differences in taurine excretion in the mouse (Harris & Searle, 1953) have been traced to intrinsic differences in its renal transport (Chesney *et al.*, 1976); studies with renal cortex slices prepared from low- and high-taurine excreting mice suggest that the defect is not located in the basolateral membrane. Since net reabsorption of amino acids *in vivo* is determined by events at the luminal membrane, we examined taurine transport in brush-border-membrane vesicles prepared from rat kidney cortex. Our findings are complementary to a recent description of β -alanine transport in a similar preparation (Hammerman & Sacktor, 1978), and they may be pertinent to our understanding of taurine uptake and storage by other tissues.

Materials and Methods

Brush-border-membrane vesicles were isolated from rat kidney cortex by the method of Booth & Kenny (1974), with minor modifications (Tenenhouse & Scriver, 1978). Purity of the preparation was evaluated by the enzyme markers alkaline phosphatase and maltase, whose activities in the membrane

preparation were approx. 6–10 times those of cortex homogenates. Electron microscopy revealed vesiculation of the membranes. Uptake of taurine was measured by the Millipore-filtration technique (Aronson & Sacktor, 1975; Tenenhouse & Scriver, 1978). Freshly prepared membrane suspensions ($40\text{--}80\ \mu\text{g}$ of protein/incubation) were incubated with buffered mannitol medium, pH 7.4, containing 300 mM-mannitol, 20 mM-Tris/Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and [^{14}C]-taurine ($10^6\text{d.p.m.}/100\ \mu\text{l}$). Mannitol was replaced with iso-osmotic NaCl or KCl when studying ionic effects on taurine uptake. Radioactivity bound non-specifically to the Millipore filters in the absence of membranes ($<1\ \text{pmol}$ of taurine/filter) was subtracted from the counts obtained with incubated membrane samples when calculating net uptake by vesicles. Protein was determined by the method of Lowry *et al.* (1951) after digestion for 15 h in 0.5 M-NaOH. Each experiment was performed twice in triplicate.

Male Long-Evans rats (175–200 g) were obtained from Canadian Breeding Farms, Montreal, Que., Canada; Millipore filters (type HA; $0.45\ \mu\text{m}$) were from Millipore Corp., Bedford, MA, U.S.A.; Hepes was from Sigma Chemical Co., St. Louis, MO, U.S.A. [^{14}C]Taurine (sp. radioactivity 114 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and reagent-grade chemicals were from standard commercial sources.

Results and Discussion

Since taurine is an inert metabolite in rat kidney (Chesney *et al.*, 1976), it is a particularly useful probe of the transport process under investigation. First, we confirmed that net uptake of taurine represents transport into vesicles rather than binding to membranes. To show this, we modified the osmolarity of the external incubation medium with sucrose, an impermeant disaccharide that cannot be hydrolysed

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Fig. 1. Uptake of taurine ($44 \mu\text{M}$) by rat renal brush-border-membrane vesicles incubated in various media (a) and effect of the K^+ -diffusion potential on the uptake of taurine ($44 \mu\text{M}$) by rat renal brush-border-membrane vesicles (b)

(a) The media contained: 100mM-NaCl and buffered 100mM-mannitol ($\bullet$); 100mM-KCl and buffered 100mM-mannitol ($\circ$); 100mM-NaCl and buffered 100mM-mannitol after preincubating the vesicles in the same medium for 90 min ($\blacktriangle$). In (b), vesicles were preincubated in 50mM-KCl and buffered 200mM-mannitol. Uptake was measured in medium containing 100mM-NaCl and buffered 100mM-mannitol in the presence ($\bullet$) and absence ($\circ$) of valinomycin ($8 \mu\text{g}/\text{mg}$ of protein). In (a) and (b), points represent the mean $\pm$ S.E.M. for normalized data ($n = 4$ for each point). Data were obtained from two experiments with duplicate determinations.

by rat kidney (Silbernagl, 1977). Uptake of taurine at equilibrium was inversely proportional to the osmolarity of the medium and, by implication, directly proportional to intravesicular volume. This relationship was observed in the presence or absence of Na^+ . Extrapolation to infinite osmolarity indicated that binding to membranes accounted for less than 10% of net uptake by vesicles at equilibrium. The estimated intravesicular volume at equilibrium and at 300m-osmol was $0.9 \mu\text{l}/\text{mg}$ of protein.

Taurine transport is concentrative in the presence of a Na^+ gradient. An early overshoot during time-dependent uptake and 20–30-fold stimulation of the initial rate of uptake were both observed in the presence of a large initial Na^+ gradient (extravesicular Na^+ greater than intravesicular Na^+ at zero time) (Fig. 1). When vesicles were preincubated in 100mM-NaCl and uptake was measured in 100mM-NaCl, the overshoot phenomenon was not observed. Nonetheless, initial uptake of taurine under these conditions exceeded that observed in an iso-osmotic medium containing either K^+ or mannitol. Accordingly, interaction of taurine with the transport process and net uptake against its own concentration gradient are both Na^+ -dependent.

Taurine transport by renal brush-border-membrane vesicles is driven by the transmembrane potential difference. Vesicles were preincubated with KCl and taurine uptake measured in 100mM-NaCl (external concentration) in the presence, or absence, of valinomycin (Fig. 1). Overshoot in the presence of the K^+ ionophore was twice that in its absence.

Table 1. Effect of various compounds on taurine uptake by the Na^+ -dependent system in rat renal brush-border-membrane vesicles

The concentration of [^{14}C] taurine at zero time in the external medium was $37 \mu\text{M}$. The incubation period was 30s. The inhibitor was present at 4mM. Specific uptake on the Na^+ -dependent system was determined by subtracting uptake in the absence of Na^+ from uptake in its presence. Net uptake of taurine in the absence of inhibitor is designated 100% (control). The results are means $\pm$ S.E.M. for six determinations.

Test substance	Relative rate of taurine uptake (%)
Control	100
Taurine	5.4 ± 1.5
Hypotaurine	0.7 ± 0.4
β -Alanine	8.4 ± 1.0
L-Alanine	25.2 ± 1.8
L-Proline	32.0 ± 1.3
D-Glucose	34.6 ± 2.6
γ -Aminobutyric acid	46.4 ± 3.1
β -Aminoisobutyric acid	81.0 ± 5.7
L-Aspartic acid	84.4 ± 2.8
Carnosine (β -alanine-L-histidine)	95.0 ± 1.3

Since the intravesicular space is hyperpolarized by valinomycin under these conditions, we presume that the transmembrane potential difference (inside negative) and an Na^+ gradient can both drive taurine transport in brush-border-membrane vesicles. The possibility that taurine entry was linked to K^+ exit,

Fig. 2. Effect of other amino acids on taurine uptake. Concentration-dependent uptake of taurine by rat renal brush-border-membrane vesicles was measured at 30s. Lineweaver-Burk transformations of uptake rates on the Na⁺-dependent carrier-mediated system are shown. The inhibitor was present at 1 mM.

independent of effects on potential difference, was not investigated.

Taurine transport is concentration-dependent. In the absence of Na⁺, uptake was directly proportional to taurine concentration over the range 30–400 μM. This Na⁺-independent component of taurine transport might be diffusional, or it might be a carrier-mediated process that saturates only at very high concentrations of the substrate. The Na⁺-dependent component of taurine uptake saturated at approx. 100–200 μM-*taurine*. Although net uptake of taurine varied between experiments, the apparent *K_m* remained reasonably constant. Five experiments, each performed in triplicate, yielded an apparent *K_m* of 16.8 ± 2.4 μM (mean ± S.E.M.) and a *V_{max}* of 222.8 ± 34.4 pmol/mg of protein per 30s. The apparent *K_m* for taurine uptake is in close agreement with the corresponding value for β-alanine in rabbit renal brush-border-membrane vesicles (Hammerman & Sacktor, 1978), and with the value for the high-affinity Na⁺-dependent taurine-transport system in rat brain (Hruska *et al.*, 1978; Lombardini, 1978). We found no evidence for a 'high-*K_m*' taurine-transport component in our kidney membrane preparation, although this has been observed in the rodent kidney cortex slice (Chesney *et al.*, 1976).

Taurine is transported on a β-amino acid-selective system in renal brush-border membranes. Hypo-taurine (the sulphinic acid analogue of taurine) and β-alanine (the carboxylic acid analogue) were potent inhibitors of Na⁺-dependent taurine uptake (Table 1). A similar finding has been reported for rat brain cortex slices and brain synaptosome-membrane fractions (Hruska *et al.*, 1978; Lombardini, 1978) and for blood platelets (Gaut & Nauss, 1976). Other substances inhibited taurine uptake, but to a lesser

extent; they include several α-amino acids and D-glucose. Exclusion of the dipeptide, L-carnosine (β-alanyl-L-histidine) from the renal transport system is in keeping with previous observations (Nutzenadel & Scriver, 1976).

β-Alanine is a competitive inhibitor of taurine uptake by brush-border-membrane vesicles (Fig. 2). The apparent *K_m* of taurine in the presence of β-alanine was 150–300 μM and the *K_m* in the absence of β-alanine was 18 μM, a value comparable with that reported above. We also examined the inhibitory effect of L-proline on taurine uptake, because net taurine reabsorption is decreased *in vivo*, in the hyperprolinaemic (PRO/Re) mouse (Chesney *et al.*, 1976). The imino acid was not a competitive inhibitor of taurine uptake by the membrane preparation (Fig. 2). The inhibition observed with L-proline may reflect dissipation of the Na⁺ gradient during its own uptake. A similar mechanism may explain inhibition by other substances (Table 1) whose own uptake is unlikely to be accommodated by the taurine system.

Our findings indicate that taurine is transported on a β-amino acid-preferring system in mammalian renal brush-border membranes. Our evidence for this substrate-selective system corroborates that of Hammerman & Sacktor (1978). The potential relevance of these observations for other tissues, such as heart, is of interest since a mechanism exists to modify cellular concentrations of taurine through specific modulation of its transport system (Huxtable & Chubb, 1977).

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