TAURINE SECRETION IN TOADFISH (OPSANUS TAU) KIDNEY: TRANSPORT IN THE INTACT KIDNEY AND IN BRUSH BORDER MEMBRANE VESICLES

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In vivo clearance studies have shown that taurine is secreted in the kidneys of several marine teleosts (Schroeck et al., Am. J. Physiol. 242:R64-R69, 1982). Since taurine appears to be accumulated in the renal tubule epithelium via a Cl-dependent Na^+-taurine-cotransport mechanism in the basolateral membrane, taurine movement across the luminal membrane occurs down its chemical gradient. However, vesicle studies have given evidence for a taurine transport mechanism in the brush border membrane also dependent on external Na^+ (King et al., J. Comp. Physiol. B 155:185-193, 1985). King et al. have suggested that this Na^+-symport system could operate in the secretory direction if the cell/lumen taurine gradient exceeds the lumen/cell Na^+ gradient. In order to substantiate this model we studied taurine transport in the aglomerular kidney of the toadfish, which could be expected to have a more effective secretory mechanism than the glomerular flounder kidney.

Toadfish of mixed sex, weighing 200-600 g, were obtained from the Marine Biological Laboratory Woods Hole. For determination of blood and urine taurine concentrations, the animals were anesthetized with MS 222 and the bladders were catheterized with PE50 tubing. The end of the catheter was fixed below the fish so that the urine drained from the bladder as fast as it entered from the ureters. Blood was drawn from a caudal vessel, centrifuged and the plasma (and urine) analyzed for taurine by automatic amino acid analysis. Brush border membranes for transport studies were isolated using the calcium precipitation method as described by Eveloff et al. (Am. J. Physiol. 237:F291-F298, 1979) and King et al. (J. Comp. Physiol. B 155:185-193, 1985), with minor modifications. [^{3}H]-taurine uptake was followed by a rapid filtration technique (Eveloff et al., Am. J. Physiol. 237:F291-F298, 1979). Protein determination and enzymatic characterization of the membranes are described in an accompanying paper by R. Kinne et al. (MDIBL Bull. 1985).

The toadfish kidney secretes taurine from blood into urine. The plasma and urine taurine concentrations were 0.035±0.005 and 0.138±0.041 mM, respectively, resulting in an U/P ratio of 3.8±0.9 (mean ± S.E., n = 6). Tissue taurine concentration was approximately 3 mM. Thus, as found previously in other marine fishes, taurine is accumulated from blood into the renal cells against a concentration gradient and then moves downhill from the cells into the tubular lumen.

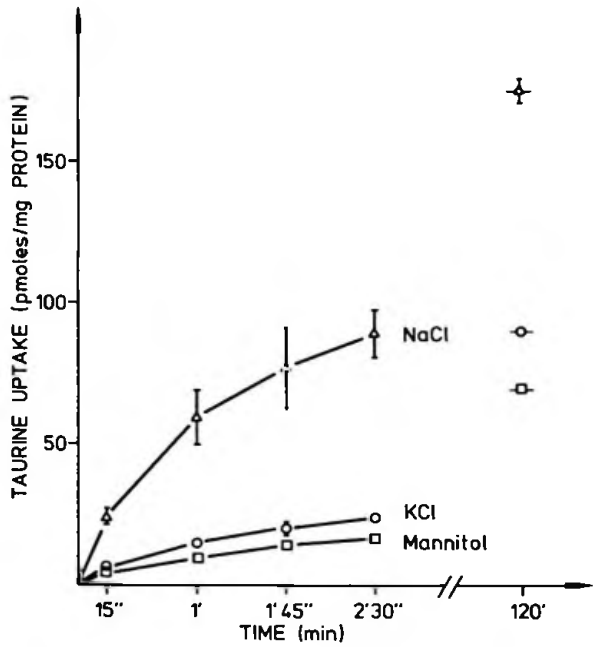

Figure 1a.-- Cation requirement of taurine uptake into toadfish renal brush border membrane vesicles. Values are means of 2 experiments or means ± S.E. (n = 3 or n = 4). Incubation media contained 20 mM Tris-HEPES, pH 8.2, 50 mM mannitol, 2 mM $CaCl_2$, 0.1 mM [^{3}H]-taurine, and 71 mM NaCl (Δ); 71 mM KCl (o) or 142 mM mannitol (□).

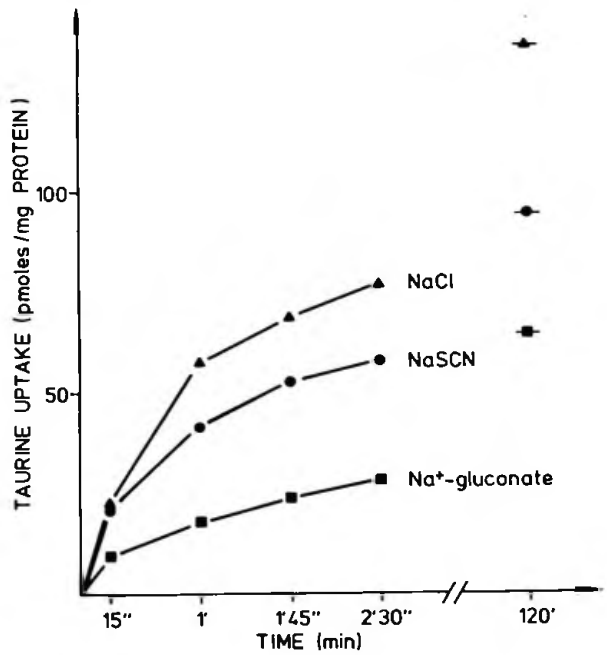

Figure 1b.-- Effect of various anions on taurine uptake by toadfish renal brush border membrane vesicles. Values are means of 2 experiments. Incubation media contained 20 mM Tris-HEPES, pH 8.2, 50 mM mannitol, 2 mM $CaCl_2$, 0.1 mM [^{3}H]-taurine, and 71 mM NaCl (▲); 71 mM NaSCN (●) or 71 mM Na^+-gluconate (■).

Fig. 1a shows taurine uptake by toadfish renal brush border membrane vesicles. The uptake exhibited a marked sodium dependence. At 15 s, uptake in a 71 mM NaCl gradient was 3.7 times higher than in an equal KCl gradient and 5.3 times higher than in a 142 mM mannitol medium. Na^+-independent uptake rates were so low, that even after 120 min the difference between uptake in NaCl controls and in KCl or mannitol medium, respectively, was still significant. Tracer replacement experiments are shown in Table 1. Addition of 5 mM nonlabeled taurine significantly decreased Na^+-dependent [^{3}H]-taurine transport. Uptake during the first 2.5 min averaged 33.3±3.7% of control values in a NaCl medium, whereas the Na^+-independent [^{3}H]-taurine transport was not affected.

Table 1.-- [^{3}H]-taurine uptake into toadfish renal brush border membrane vesicles: inhibition by L-taurine and β-alanine

	taurine uptake (pmoles/mg protein)				
	0.25 min	1.0 min	1.75 min	2.5 min	120 min
NaCl: control (4)	27.0±2.0	67.0±7.6	87.8±9.5	105.1±10.0	160.8±14.9
plus 4.7 mM tau (3)	10.4±1.5	22.0±1.4	28.5±2.8	31.2± 1.9	78.9± 5.3
	p < 0.01	p < 0.01	p < 0.01	p < 0.001	p < 0.01
plus 4.7 mM β-ala (2)	6.0±3.6	12.5±5.1	20.4±8.4	20.7± 7.2	75.0± 9.6
	p < 0.01	p < 0.01	p < 0.01	p < 0.01	p < 0.01
KCl: control (4)	6.9±1.4	15.2±0.6	19.2±2.0	22.9± 1.7	90.7± 0.6
plus 4.7 mM tau (3)	10.3±2.5	16.2±2.5	21.3±2.7	24.0± 2.4	67.2
	NS	NS	NS	NS	NS
plus 4.7 mM β-ala (1)	6.7	13.1	14.3	16.3	76.2

The values are means ± S.E., the number of experiments is shown in parentheses. The incubation media contained 20 mM Tris-HEPES, pH 8.2, 50 mM mannitol, 2 mM $CaCl_2$, 0.1 mM [^{3}H]-taurine and 71 mM NaCl or 71 mM KCl. Taurine or β-alanine was added to give a final concentration of 4.7 mM. Significance of differences between two means was calculated using the Student t-test for group data and is indicated by P values. NS = not significant; tau = taurine; β-ala = β-alanine.

Since it is known that in a variety of tissues taurine shares the Na^+-dependent transport system with other β-amino acids, we tested the effect of 5 mM β-alanine on taurine uptake (Table 1). β-alanine inhibited the Na^+-dependent transport by about 80% at 2.5 min. Since [^{3}H]-taurine flux in a β-alanine containing medium was even lower than in the presence of an equal concentration of nonlabeled taurine, the affinity of the carrier for β-alanine appears to be higher than for taurine. The Na^+-independent taurine uptake remained unaffected by β-alanine.

In a third series of experiments we investigated the anion dependency of taurine transport (Fig. 1b). When a NaCl gradient across the brush border membrane was replaced by a NaSCN or a Na^+-gluconate gradient an inhibition of uptake after 2.5 min by 20% or 62%, respectively, was observed.

Conclusions

The finding that the brush border of the aglomerular fish Opsanus tau contains a sodium-taurine cotransport system similar to the one found in the brush border of the glomerular fish Pseudopleuronectes americanus supports the hypothesis presented previously that the sodium-taurine cotransport system is involved in taurine secretion rather than taurine absorption in the proximal tubule. The operation of this cotransport system in the secretory direction might also have the advantage that, simultaneously with taurine, sodium is secreted into the tubular lumen. The intratubular sodium can lead to the movement of water into the lumen of the tubule, thereby generating the fluid necessary to excret the secreted solutes.