RENAL HANDLING OF TAURINE IN THE DOGFISH SQUALUS ACANTHIAS

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Taurine plays an important role in osmoregulation in elasmobranch fishes. It is found in high concentrations in the heart, brain and erythrocytes of the fish when they are in sea water. During acclimation to dilute seawater, taurine is released from these tissues to help maintain a constant cell volume (T.A. Boyd et al, J. Exp. Zool. 199, 435-442 (1977); R.P. Forster et al., Bull. Mt. Desert Is. Biol. Lab. 18, 1-4 (1978)). Since taurine is not metabolized in elasmobranchs (P. King and L. Goldstein, Bull. Mt. Desert Is. Biol. Lab. 17, 16-19 (1977)) the fishes must have some way of excreting taurine rapidly when they enter a dilute environment. King et al, have previously found that it is excreted at a greater rate in skates acclimated to dilute seawater compared to fish maintained in normal seawater. In the present study we examined the mechanism of excretion of taurine by the dogfish kidney and the effect of environmental dilution on this excretion.

Adult, female spiny dogfish weighing about 5 kg (Squalus acanthias) were caught on set-lines in Frenchman Bay, Maine. They were maintained without food in running seawater in a 3 x 5 m tank and were used 3-6 days after capture. One group of dogfish was adapted to 70% seawater by a daily 10% increase in the freshwater inflow for 3 days. Renal clearance studies were then performed after the fish had acclimated to 70% seawater for one day. The urinary papilla was catheterized and 10 ml of 5% inulin in balanced elasmobranch solution was injected into tail muscle 12 hours before the start of the experiment. Two 4-hour samples were then collected in balloons tied to the indwelling catheter. Blood was withdrawn from the caudal vein at the beginning and end of each urine collection. Inulin in urine and plasma was determined by the resorcinol method. Taurine in urine and plasma was determined according to the colorimetric analysis of Pentz et al, J. Biol. Chem. 228, 433 (1957). In two animals taurine was assayed with an automatic amino acid analyzer.

Transport of taurine by renal cells was studied in vitro with kidney slices. Slices were incubated in balanced elasmobranch solution (R.P.Forster et al, Comp. Biochem. Physiol. 43A, 3-12 (1972) at 15°C for 1 hour under various conditions. The medium generally contained 0.1 mM taurine, 0.1 μCi/ml ^{14}C-taurine, 5 mM glucose and was gassed with 1% CO_2-99% O_2 mixture for at least 10 minutes prior to incubation. Tissue slices weighed between 20-50 mg. Radioactivity was assayed by liquid scintillation counting. The incubation medium was assayed with Aquasol. The tissue slices were digested with Protocol and measured in PPO, POPOP-toluene cocktail. Student's t-test was employed to establish the level of significance between two means.

Female dogfish in 100% seawater showed considerable variation in plasma and urine taurine concentrations (Table 1). Despite this, U/P ratios for taurine were always greater than U/P ratios for inulin ($P<0.01$), although there was still considerable variation from fish to fish. Nevertheless, it is clear that taurine was secreted by the dogfish kidney.

In 70% seawater plasma and urine taurine concentrations were more uniform. Plasma taurine concentration increased significantly ($P < 0.001$) in diluted fish as a result of taurine losses from tissues. U/P ratios for taurine remained significantly higher than U/P ratios for inulin ($P < 0.01$) indicating that secretion persisted in fish adapted to dilute seawater. Total taurine excretion increased by the factor of 3.7 in fish in 70% seawater (Table 2). This increase in excreted taurine was due in large part to a rise in filtration, but secretion of taurine was significantly increased ($P < .05$) as well. In a separate experiment we determined that the taurine kidney/plasma ratio was 9.9 and the urine/kidney ratio was 0.5. This suggested that plasma taurine could be accumulated from the blood side of the tubular cells and then diffuse down its concentration gradient into the tubular lumen. Therefore, we examined examined the characteristics of taurine transport by kidney slices incubated in elasmobranch Ringer's solution.

Table 1. Taurine secretion by the kidney of the dogfish, Squalus, Acanthias

	ENVIRONMENT	
	100% Seawater	70% Seawater
Plasma taurine (mM)	0.91 ± 0.21	2.46 ± 0.10*
Urine taurine (mM)	7.54 ± 1.73	7.01 ± 0.61
U/P taurine	7.81 ± 1.08	2.87 ± 0.27*
U/P inulin	3.94 ± 0.38	1.88 ± 0.18*
$\frac{\text{U/P taurine}}{\text{U/P inulin}}$	2.32 ± 0.35‡	1.63 ± 0.20‡

Values are means ± S.E. for 10-12 fish in 100% seawater and 9 fish in 70% seawater.

*Significantly different from corresponding value in the same fish in 100% seawater.

‡Significantly different from 1.00.

Table 2. Adaptation of renal taurine excretion in dogfish maintained in diluted seawater

	ENVIRONMENT	
	100% Seawater	70% Seawater
GFR (ml/kg x hr)	2.07 ± 0.32	3.14 ± 0.19
Urine flow (ml/kg x hr)	0.59 ± 0.11	1.81 ± 0.22*
Taurine filtered (mmols/kg x hr)	1.27 ± 0.23	7.77 ± 0.67*
Taurine secreted (mmols/kg x hr)	2.18 ± 0.63	5.18 ± 1.48*
Taurine excreted (mmols/kg x hr)	3.45 ± 0.82	12.95 ± 1.87*

Values are means ± S.E. of 10 fish in 100% seawater and 9 fish in 70% seawater.

*Values are significantly different from corresponding values in fish in 100% seawater.

Uptake of ^{14}C-taurine by dogfish kidney slices was found to be linear for 3 hours. The tissue to medium ratio for taurine after 3 hours was approximately 6. Experiments with various incubation conditions were done using a 1-hr incubation period (Table 3). Sodium-free incubation medium, chloride-free incubation medium, ouabain and 2.4 dinitrophenol inhibited taurine uptake. Potassium-free medium, probenecid, β-alanine, $Na_2S_2O_3$ and furosemide had no significant effect on taurine uptake. Na_2SO_4 increased taurine uptake significantly ($P < 0.05$). Accumulation of ^{14}C-α-methyl glucoside (not shown), which represents mainly diffusion of this compound into the intercellular space and uptake via the luminal side of the cell, was negligible compared to taurine. The tissue/medium ratio of this compound was 0.38 ± 0.022 (n=8). Thus it appears the taurine is accumulated mainly by active uptake on the basolateral surface of the cell.

Sodium-free incubation medium produced the biggest inhibition of taurine uptake. The tissue/medium ratio was 0.27 under these conditions, which probably represents taurine localized in the extracellular spaces of the slice.

Table 3. Taurine S/M ratios in dogfish kidney slices incubated in vitro

Medium	Inhibitor	S/M ratio
EIM*	--	1.77 ± 0.18(9)
EIM (-) Na^+ ‡	--	0.27 ± 0.02(6)+ ‡
EIM (-) K^+ ‡	--	1.59 ± 0.10(6)
EIM (-) Cl^- ‡	--	0.75 ± 0.05(3)+
EIM	Ouabain (0.1mM) no preincubation	1.10 ± 0.08(6)+
EIM	Ouabain (0.1mM) 40 min preincubation	0.67 ± 0.03(3)+
EIM		
EIM	2,4 dinitrophenol (0.1mM)	0.93 ± 0.05(6)+
EIM	Furosemide (0.1mM)	1.56 ± 0.11(3)
EIM	Probenecid (1mM)	2.55 ± 0.36(4)
EIM + Na_2SO_4 (10mM)	--	2.34 ± 0.27(9)+
EIM + $Na_2S_2O_3$ (10mM)	--	1.92 ± 0.19(6)

Values are means ± S.E. Numbers of slices in parentheses.

*EIM = elasmobranch incubation medium.

+Significantly different from value in unmodified EIM.

‡Na^+ replaced by choline, K^+ replaced by Na^+, Cl^- replaced by mixture of $SO_4^=$ and mannitol.

S/M = slice to medium ratio (dpm · g slice^{-1}/dpm · ml medium^{-1}).

Chloride-free incubation medium and preincubation with ouabain both inhibited taurine uptake partially, as indicated by the ratios of 0.75 and 0.67. This is in contrast to the findings in the rat by Awapara and Berg, in "Taurine" ed. R. Huxtable and A. Barbeau, Raven Press, N.Y. (1976) where ouabain did not inhibit taurine accumulation by kidney slices, so a Na-dependence exists. Inhibition of all oxidative phosphorylation by 2,4 dinitrophenol resulted in a tissue/medium ratio of 0.93 indicating that taurine can enter the cell under these conditions but no concentration gradient can be established. The stimulation of taurine transport by sulfate and inhibition by removal of chloride suggest that taurine may be transported into the cell by a low specificity anion transport system. Supported by NSF PCM 75-14322 and NIH HLO4457. Dr. H. Schröck was supported by a stipendium from the Deutsche Forschungsgemeinschaft (West Germany) No. Schr. 215/1.

ACTIVITIES OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE, ALDOLASE, LACTATE DEHYDROGENASE, AND ISOCITRATE DEHYDROGENASE IN SCULPIN AND DOGFISH CORNEA AND MUSCLE

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In mammalian species, the cornea comprises the major refractive component of the eye's optical system. Thus, its transparency is of crucial importance. The limiting layers of the cornea (epithelium and endothelium) maintain this transparency by keeping the hydrophilic stroma at a minimum of physiologic hydration. These layers serve this function by providing 1) barriers to the movement of ions and water, and 2) metabolic pumps which actively maintain