^{3}H-ALA antifreeze peptides synthesized from antifreeze mRNA in a cell-free protein synthetic system were used for injection (Lin, J. Biol. Chem., 254: 1422, 1979; Lin and Long, Biochemistry 19: 1111, 1980). The native antifreeze peptides (NAP) were then modified by the carbodiimide reaction (Hoare and Koshland, J. Biol. Chem., 242: 2447, 1967) with methyl ester lysine substitution at the activated free carboxyl groups of the NAP to yield the cationic antifreeze peptides (CAP). The native and cationic antifreeze peptides were of similar molecular weights as evidenced by similar migration distances in a 17.5% polyacrylamide urea-SDS electrophoresis gel. The isoelectric point of the NAP was 4.5 → 4.9 while the isoelectric point of the CAP was 6.0 - 7.0.

Renal clearance values of PEG and the antifreeze peptides are presented in Table 1. The fractional

Table 1.--Renal Clearances of Polyethylene Glycol (PEG), Native Antifreeze (NAP) and Cationized Antifreeze Peptides (CAP) in the Winter Flounder Collected from Frenchman Bay, Near MDIBL, Maine

Substance Injected	pI	n	Weight (kg)	Urine Flow Rate (ml/hr/kg)	U/P	Clearance	Fractional Clearance
PEG	--	12(107)*	.39(.03)**	.41(.11)	2.97(.53)	0.78(.09)	1.00
NAP	4.5-4.9	3(24)	.39(.07)	.59(.07)	0.06(.01)	0.03(.01)	.043(0.007)
CAP	6.0-7.0	4(40)	.35(.01)	.74(.18)	0.51(.09)	0.29(.02)	.370(0.029)

Experimental temperature 13°C.

*n of fish (# of clearance periods).

**(± S.E.)

clearance of the NAP reveals that only 4% of the NAP passes into the bladder urine. It is possible that filtration might have been followed by reabsorption at the proximal tubule, but the reabsorption process is energetically expensive and this process is not likely to occur since the winter flounder does not compensate metabolically in the cold. Upon addition of lysine to the peptides there was an 8.5 fold increase in the fractional clearance. This increase in clearance strongly suggests that charge-charge interaction plays an important role in the conservation of antifreeze peptides in the flounder kidney.

Our results also indicate that the charge dependent conservation of the native and cationic antifreeze peptides is similar to the renal handling of anionic, neutral and cationic derivatives of albumin (Purtell et al., Kid. Int., 16: 366, 1979) and horseradish peroxidase (Rennke et al., Kid. Int. 13: 324, 1978) in the rat. This research was supported by NIH predoctoral training Grant 5T32GM143 (To DHP) and NSF Grant PCM 77-25166 (to ALD).

CHARACTERIZATION OF TAURINE UPTAKE BY INCUBATED SLICES OF DOGFISH KIDNEY (SQUALUS ACANTHIAS)

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Taurine not only is a unique amino acid considering its chemical composition (2-aminoethanesulfonic acid) but it was recently found to be handled by the kidneys in an unusual way as well. Free amino acids are generally filtered by the glomerulus and are reabsorbed subsequently. Additionally, net tubular excretion of taurine was demonstrated for the first time in the dogfish (Schröck et al. Bull. Mt. Desert Is. Biol. Lab., 19:41-43, 1979).

This unique behavior of an amino acid initiated these experiments which attempt to describe the basic process underlying taurine uptake by dogfish kidney slices.

Dogfish (BW - 5.8 ± 0.2 kg, n = 24) were caught by set-lines in Frenchman Bay, Maine and kept in a live car without food. Only female fish were used.

Uptake of taurine by renal cells was studied in vitro with dogfish thin (0.5 mm) kidney slices. Slices were incubated in balanced elasmobranch solution (Forster et al., Comp. Biochem. Physiol. 43A, 3-12, 1972), at 15± 1°C for 1 or 2 hours under various conditions. Except with the saturation experiments, the incubation medium usually contained 1 mM taurine, 0.1 μCi/ml ^{14}C-taurine, 5 mM glucose and was gassed with a 1% $C0_2$ - 99% 0_2 mixture for at least 10 minutes prior to incubation. Tissue slices (20-60 mg) were weighed after the end of each incubation. Extracellular space of incubated kidney slices was estimated with ^{14}C-PEG. Radioactivity was assayed by liquid scintillation counting. The incubation medium was measured in Aquasol. The tissue slices were digested overnight with Protosol and measured in a PPO, POPOP-toluene solution.

Water content of incubated kidney slices was determined by weighing wet and then after drying at 80°C in an oven overnight. Taurine uptake by the kidney cells was expressed as μM tau/g cell H_20/hour which includes a correction made for taurine present in the extracellular space.

^{14}C-taurine and ^{14}C-PEG were purchased from New England Nuclear. DIDS (4,4 '-diisothiocyano stilbene - 2,2'-disulfonic acid), and SITS (4 acetamido - 4'-isothiocyano stilbene - 2,2'-disulfonic acid) were purchased from Pierce. All other chemicals came from Sigma or Fisher. Student's t-test was used for test statistics.

Time course and viability: When kidney slices were incubated with 1 mM taurine, uptake was linear over 4 hours. The slice to medium ratio for taurine was 3.6 after 4 hours, thus an accumulation of taurine into the slice occurred. The water content of the incubated kidney slices (3.74 ± 0.08 g H_20/g dry wt., n = 15) as well as the estimated extracellular space (0.36 ± 0.02 g extracell. H_20/g wet wt., n = 15) did not change significantly during the 4 hour incubation period. This, together with the linearity of taurine uptake, establishes the viability of the kidney slices for the incubation times of 1 or 2 hours used in the experiments reported below.

Saturation of uptake: Incubation of kidney slices with medium taurine concentrations ranging from 1 mM to 10 mM shows incomplete saturation when uptake (μM tau/g cell H_20/h) is plotted against medium taurine concentration. The Lineweaver-Burk plot yields a Km of 8.8 mM; V_{max} is 21 μM tau/g cell H_20/h.

Effects of metabolic inhibition: (Table 1) Incubation of dogfish kidney slices with 0.1 mM 2,4-DNP, 1mM NaN_3, or in the cold at 4°C inhibited uptake of taurine significantly when compared to controls, thus establishing the dependency of taurine uptake, directly or indirectly on the supply of metabolic energy.

Effects of inorganic ions: Low Na, low K, and low Cl concentrations inhibited taurine uptake into the kidney slice cells significantly. High K, with a concentration double that of normal plasma concentrations did not have an effect on taurine uptake, indicating that the electric potential across the cell membrane is not important for uptake. Ouabain and low Na both produced the biggest inhibition; taurine uptake was almost zero, indicating that Na is linked directly or indirectly to the entry of taurine into the cell. The inhibition of taurine uptake by ouabain is in contrast to the findings in the rat, where ouabain did not inhibit taurine uptake into incubated kidney slices while the uptake was Na-dependent (Awapara and Berg, in "Taurine" ed. by Huxtable and Barbeau. Raven Press, N.Y., 1976). Thiosulfate and sulfate did not inhibit taurine uptake significantly.

Effects of analogues: α-AIBA and γ-ABA and glycine all at 10 mM, did not inhibit taurine uptake. β-alanine (10-20 mM) inhibited taurine uptake significantly. Hypotaurine inhibited taurine uptake, which is to be expected because it is very close, chemically, to taurine itself.

Table 1.--Intracellular Uptake of Taurine into Incubated Dogfish Kidney Slices, given as μM Tau/g Cell H_2O/hr

Treatment	Control	n	Experimental	n	t-test	Uptake (in % of control)
low Na	2.67 ± 0.17	13	0.03 ± 0.02	13	P<0.001	1
low K	3.27 ± 0.16	13	1.75 ± 0.14	13	P<0.001	54
low Cl (NaAc)	2.67 ± 0.21	10	0.37 ± 0.02	10	P<0.001	14
low Cl (Na_2SO_4 + Mannitol)	2.59 ± 0.99	3	0.99 ± 0.14	3	P<0.005	38
Ouabain (0.1 mM)	2.81 ± 0.14	8	0.05 ± 0.00	8	P<0.001	2
2,4 DNP (0.1 mM)	3.46 ± 0.29	15	1.48 ± 0.36	15	P<0.001	43
Cold (+4°C)	3.07 ± 0.26	10	0.72 ± 0.08	9	P<0.001	23
NaN_3 (1 mM)	3.83 ± 0.25	15	1.09 ± 0.14	15	P<0.001	28
Hypotaurine (10 mM)	2.96 ± 0.32	8	0.77 ± 0.21	8	P<0.001	26
β-ALA (10 mM)	3.13 ± 0.17	23	2.20 ± 0.22	23	P<0.005	70
β-ALA (20 mM)	2.89 ± 0.18	5	1.79 ± 0.11	5	P<0.001	62
DIDS (25μM)	3.07 ± 0.16	20	4.08 ± 0.19	20	P<0.001	133

Mean ± S.E.M. Values are corrected for extracellular space. n = number of slices. From each fish 3-5 slices were taken for a single experiment. low Na = NaCl replaced by Choline-Cl, low Cl = Cl replaced by Na-Acetate or Na-Sulfate + Mannitol respectively.

Effects of other organic agents: PAH, probenecid, phlorizin, SITS, C-AMP + theophylline, and the Ca-ionophore A 23187 did not affect taurine uptake significantly suggesting that taurine transfer is not influenced by members of the PAH anionic transport system, sugar transport, C-AMP as a second messenger (and thus hormones which work through C-AMP) and Ca^{+2}. DIDS was the only compound which increased taurine uptake into incubated dogfish kidney slices significantly. This effect cannot be explained at the moment. Supported by NIH 4L04457 and NSF PCM 7921476. Dr. H. Schröck was supported by a stipendium from the Deutsch Forschungsgemeinschaft Grant Schr 215/2.

RENAL HANDLING OF TAURINE IN THE LITTLE SKATE RAJA ERINACEA AND THE WINTER FLOUNDER PSEUDOPLEURONECTES AMERICANUS

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Taurine, the sulfonate analogue of β-alanine, serves as an important osmotic agent in regulating cell volumes of certain organs in invertebrate and vertebrate marine species. It is found in high concentrations in the heart, brain and erythrocytes of the elasmobranch skates for example. During acclimation to dilute seawater brain and erythrocytes but not heart tissue release taurine and thus help maintain intracellular osmolality similar to that of interstitial fluids