Flounder gallbladder, therefore, appears to have essentially the same ion transport properties as flounder intestine, namely, a serosanegative PD, which is inhibitable by cyclic AMP and also by a reduction in medium pH, and a high degree of cation selectivity in the paracellular pathway. Gallbladder resistance is about 50% higher than intestinal resistance. Morphologically the cells also appear similar in that they are both unusually long and narrow.

IDENTIFICATION OF AN INTRACELLULAR UPTAKE SYSTEM FOR TAURINE IN ATRIAL MYOCARDIUM OF THE SKATE, Raja erinacea

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The object of the current study is to establish values for the distribution of total tissue water, and the concentrations of taurine therein, between extracellular and intracellular regions in isolated "hemi-artria" of skate hearts. This is a continuation of last summer's work attempting to characterize a carrier mediated taurine transport system in sakte myocardium. Expressing taurine concentrations on an intracellular rather than on a unit per total wet weight basis becomes particularly important when agents are used selectively to elucidate the cellular taurine transport system that simultaneously bring about a redistribution of intra- and extracellular water within the in vitro fragment during incubation (Forster, R.P. et al., Bull. Mt. Desert Is. Biol. Lab. 15: 1-4, 1977).

Taurine has been implicated in cell volume regulation in various tissues of a wide variety of invertebrates and vertebrates. In addition, its role in mammalian cardiac function derives from the observation that there is an increase in taurine uptake associated with various aspects of cardiac stress (Read, W.O. and J.D. Welty. J. Pharmacol. Exper. Therap. 139: 283-289, 1963). β-adrenergic stimulation has been shown to increase the transport capacity of taurine in rat hearts (Huxtable, R. and J. Hubb. Science 198: 409-411, 1977). Taurine transport was mediated by a β-amino acid uptake system in organ culture of fetal mouse hearts that was sodium dependent and capable of accumulating taurine against a concentration gradient (Grosso, D.S., Roeske, W.R. and Bressler, R.J. Clin. Invest. 61: 944-952, 1978).

The skate was used in this investigation because, as with all elasmobranchs, its heart has no sympathetic adrenergic supply and thus is an interesting model to test the previously suggested relationship in mammals between β-adrenergic stimulation and increased taurine influx (Burnstock, G. Pharmacol. Rev. 21: 247-324, 1969). Furthermore, the in vitro hemi-atrium preparation is an extremely viable preparation for use in transport studies generally, and Raja erinacea heart has the highest taurine values reported in any vertebrate species.

Methods

Procedure is generally the same as that we described in the 1978 Bull. Mt. Desert Is. Biol. Lab. 18: 1-4. The separated thin-walled atria were divided in half, blotted, weighed, and the separate samples then placed in Erlenmeyer flasks and preincubated in balanced isotonic medium for 15 min at 15°C. The hemi-atria were then transferred to new flasks containing elasmobranch medium and incubated for 1 hr under control conditions. In all cases, paired analyses were carried out with one hemi-atrium used as control while the other was subjected to the experimental variable. Details are included with the presentation of results. Control elasmobranch medium had the following concentration in millimoles per liter: 280 NaCl, 6 KCl, 5 $CaCl_2$, 3 $MgCl_2$, 0.5 Na_2SO_4, 1 NaH_2PO_4, 450 urea; these diluted approx. to 1 liter, and 8 $NaHCO_3$ then added dry while stirring. Five millimoles/liter of glucose were included on the day of use. Vessels were gassed with 99% O_2, 1% CO_2; pH 7.4.

Results and Discussion

The viability of the skate hemi-atrium preparation was further attested to by the uniformity of extracellular fluid space (ECS), measured as the percentage volume of distribution of [^{14}C] inulin. Table 1 demonstrates the

Table 1. Constancy of extracellular space measurements under control conditions on atria incubated 1, 2, 4, and 6 hours at 15°C, and also at 3°C

Percentage ECS			
1 hr	2 hr	4 hr	6 hr
		15°C (control)	
.336 ± .035	.356 ± .044	.399 ± .046	.362 ± .044
SEM = .011 (11)	SEM = .011 (18)	SEM = .020 (6)	SEM = .031 (3)
	t = 1.27 (NS)	t = 1.63 (NS)	t = 0.86 (NS)
		3°	
.329 ± .022	.288 ± .078	.311 ± .043	.339 ± .043
SEM = .013 (4)	SEM = .039 (5)	SEM = .028 (5)	SEM = .022 (5)
	t = 1.00 (NS)	t = .0486 (NS)	t = 0.324 (NS)

Student's t values for 2 and 4 hr express the lack of significant difference between each datum and that preceding it. The 6 hr value in the control series evaluates the difference between it and the original 1 hr ECS. Number of atria per group is shown in parentheses following the standard error of the mean. Percentage ECS includes ± standard deviation.

lack of significant change in controls at 15°C and 3°C over incubation periods of 1, 2, 4, and 6 hours. The atria beat continually during incubation at 15°C, the temperature of ambient sea water at the M.D.I. Biol. Laboratory, but the beat stopped at 3°C.

In contrast, ECS was profoundly affected by Na depletion, i.e. gradual replacement of NaCl in the medium with choline chloride (Table 2).

Table 2. Extracellular space measurements in skate atria as affected by diminishing amounts of sodium in the in vitro medium at 1 and 2 hr incubation periods

	Percentage ECS	
	1 hr	2 hr
280 mM Na (control)	.354 ± .057 SEM = .018 (25)	.356 ± .044 SEM = .011 (18)
140 mM Na	.311 ± .048 SEM = .028 (4) t = 1.30; P < .5 NS	.325 ± .022 SEM = .013 (4) t = 1.85; P < .1 NS
70 mM Na	.264 ± .019 SEM = .011 (4) t = 4.27; P < .001	.255 ± .021 SEM = .012 (4) t = 6.27; P < .001
35 mM Na	.258 ± .011 SEM = .008 (3) t = 4.86; P < .001	.277 ± .003 SEM = .013 (4) t = 4.64; P < .001
0 mM Na	.170 ± .037 SEM = .022 (4) t = 6.53; P < .001	.236 ± .025 SEM = .014 (4) t = 6.74; P < .001

The percentage of the change from control ECS, ± standard deviation, was evaluated by Student's t test. The number of values is within parentheses following standard error of the mean.

A significant decrease was first noted with 70 mM Na and at zero Na, ECS diminished to approximately 50% of control values with 1 hr incubation. ECS increased with time, as noticed particularly in the rise for 17% to more than 23% with total depletion in medium between the 1-2 hr incubation periods. Furthermore, ECS was markedly reduced (61%; $p << .001$) with ouabain (10^{-4}M) during a 1-hr incubation period, i.e., from control values of 35% to 14%. On the other hand sodium azide (10^{-2}M) had no significant effect on percentage ECS.

Figure 1 shows the time course of taurine uptake by atrial myocardium under control conditions at 15°C, and also in the cold at 3°C. Taurine uptake was corrected for ECS equilibration (34.7% ECS), and linear for this 6 hr study with 0.1 mM taurine in the medium. Taurine was accumulated against a concentration gradient of 65.5 μmoles/gram wet wt

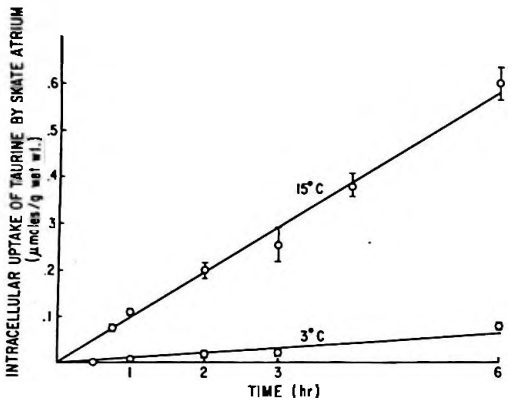

Figure 1. Time course of taurine uptake. Individual atria were incubated for 1-6 hours at both control temperature of 15°C and in the cold (3°C) in medium containing 0.1mM [^{14}C]taurine. Taurine uptake was corrected for passive ECS contribution (34.7% at 15°C and 31% at 3°C). Each value represents mean ± SEM. Sample size varies between 3 and 40. The lines are "best-fit" according to a least-squares linear regression analysis, 15°C control $y = 0.096x$.

skate atrium (Forster et al, Bull. Mt. Desert Is. Biol. Lab. 18: 1-4, 1977). Taurine uptake was temperature dependent as shown in the 94.5% drop from 15°C control rate at 3°C. Again, the sluggish accumulation was corrected for ECS equilibration (31%).

The dependence of taurine uptake on Na in the medium is shown in Figure 2. Skate atria were incubated under

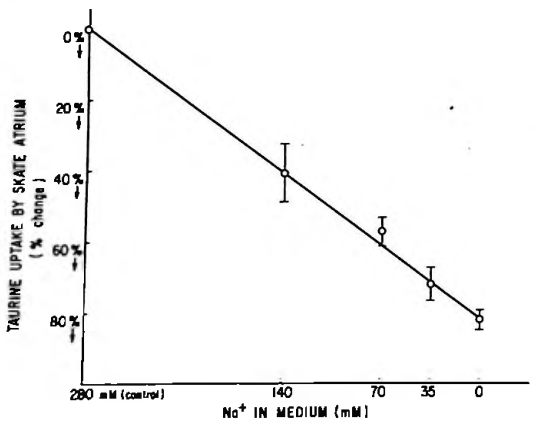

Figure 2. Na dependence for taurine uptake by skate atria using paired internal controls incubated 1 hr at 15°C. Percentage change from control was related to diminishing Na concentrations. Values expressed means ± SEM.

control conditions at 15°C for 1 hr., and [^{14}]taurine intracellular uptake was corrected for ECS contribution. NaCl was either completely omitted or substituted stepwise by choline chloride. At half control levels of Na taurine uptake dropped 40%, from .092 μmoles/g wet wt per hr to .054. The drop was linear down to an approximate 80% drop in taurine uptake (.017±.004 μmoles/g per hr; SEM = .003) with complete replacement of Na in the medium. Thus Na dependence in skate atrium is in agreement with similar findings in mammalian retina, platelets, kidney, heart slices, fetal heart, and brain which have also demonstrated that Na was required for maximal rates of taurine transport (Grosso, D.S. et al., J. Clin. Invest. 61: 944-952, 1978).

The effect of an osmotically diluted medium on the uptake rates of [^{14}C]taurine by skate atrium was tested, first, by diluting both NaCl and urea one-third to achieve an osmolality of 700 mosmoles/l and, second, by keeping the NaCl at the usual level of the control medium (280 mM) while achieving the same diluted medium observed in the plasma of environmentally diluted skates (50% sea water) by correspondingly reducing only the urea content of the in vitro medium. In internal paired controls taurine uptake in the former was reduced by 78% of control uptake and only by 46% in the dilution experiment containing "normal" Na^+ levels. The decreases are significantly different from each other with a probability P value of $< .05$ according to Student's t distribution. Thus in elasmobranchs such as Raja erinacea, where the osmolality of plasma usually drops 30% as the fish encounter diluted sea water, the osmoregulatory role of amino acids in cell volume regulation may be accounted for by Na^+ dilution, per se, rather than due entirely to some general osmolality effect on intracellular solute content. This would be particularly apt where taurine in critical tissue such as brain or heart constitutes 50% or more of the total free amino acids (Forster, R.P. et al, Comp. Biochem. Physiol. 60A: 25-30, 1978).

Ouabain (10^{-4}M) decreased control taurine uptake 41.28% ($P < .01$) corrected for ECS, in contrast to mammalian heart where in identical concentrations it actually increases taurine uptake by more than 25%. Na azide (10^{-2}M), an inhibitor of electron transport between cytochrome oxidase and molecular oxygen, decreased taurine uptake 75.23% ($P < .001$). In confirmation of last summer's work on skate atria which indicated that the taurine transport mechanism is selective for β-amino compounds, the taurine analogue β-alanine (0.5mM) diminished taurine uptake 57%, whereas related γ and α amino acids such as α-aminobutyric acid and α-aminoisobutyric acid had no significant competitive inhibitory effects. Similarly the following compounds previously shown to have a relationship between β-adrenergic stimulation and increased taurine influx in mammalian hearts; isoproterenol, dibutyryl cyclic AMP and theophylline, were ineffective in altering rates of taurine uptake. The skate, as with all elasmobranchs, lacks sympathetic adrenergic cardio-accelerator fibers to the heart (Burnstock, G. Pharm. Rev. 21: 247-324, 1969).

Conclusions

There are significant redistributions of intra- and extracellular water that result from the use of certain agents and other conditions used to characterize membrane transport systems, in this case the demonstration of a carrier mediated system for taurine in the myocardium of the skate atrium. Taking corrections for ECS content into account our in vitro studies show that taurine was accumulated intracellularly against a steep concentration gradient in atria incubated in 0.1 mM taurine by a system that was temperature dependent, required sodium, strongly inhibited by β-alanine, and unaffected by ouabain and β-adrenergic stimulation. Supported by NIH grant HLO4457.

FURTHER STUDIES OF THE EFFECTS OF PETROLEUM HYDROCARBONS ON MARINE BIRDS

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Recent laboratory and field studies with Herring gulls (Larus argentatus) and Black Guillemots (Cepphus grylle) have indicated that small oral doses of ingested crude oil cause a number of physiological aberrations in young seabirds,