CHARACTERIZATION OF TAURINE UPTAKE *IN VITRO* BY HEART OF THE LITTLE SKATE, *Raja erinacea*

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The hemi-atrium preparation is an excellent model for *in vitro* studies (Forster, R. P. et al., Bull. Mt. Desert Is. Biol. Lab. 17:11-16, 1977). Its functional viability is indicated by a spontaneous beat that persists for at least 4 hours. Also, during 2-hour control incubation periods no intracellular amino acids are released despite a very high intra- to extracellular concentration gradient, nor is the normal asymmetric transmural distribution of Na^+ and K^+ disturbed. In contrast to the ventricle, the skate atrium has little or no functional coronary circulation and its spongy organization facilitates the distribution of respiratory gasses and nutrients from ambient medium directly to the myocardial fibers which are widely disposed in an extensive extracellular fluid compartment (inulin space = 40%).

Taurine, the sulfonate analogue of β-alanine, comprises approximately 90% of the skate atrium's free amino compounds (Table 1), and its very high concentration in intracellular water (122 mM) is maintained notwithstanding an approximate 1000:1 gradient with plasma taurine. Data implicating β-adrenergic stimulation in taurine transport by heart cells of the rat (Huxtable, R. and J. Chubb, Science 198:409-411, 1977) prompted our current studies on the skate heart. Rates of taurine uptake by the perfused rat heart increased with β-adrenergic stimulation and with the administration of adenosine 3',5'-monophosphate and theophylline. Cardiac stress has also been shown to be associated with increased taurine uptake and this may be the reason why taurine is the only amino acid significantly elevated in hypertension and congestive heart failure. The skate is an interesting model to test the suggested relationship between β-adrenergic stimulation and increased taurine influx because it is, as with all elasmobranchs, totally devoid of a sympathetic adrenergic nerve supply to the heart (Young, J. Z., Q. J. Micr. Sci. 75:571-624, 1933). We wished to use this comparative feature to test the suggestion that there is a taurine transport system in the heart whose activity is regulated by the level of β-adrenergic activation, and to characterize as fully as possible *in vitro* the taurine (β-amino acid) uptake process in the skate atrium.

Methods

Raja erinacea of mixed sex with weights ranging from 1-2 kg were collected by drag net from waters off Mt. Desert Island and maintained in circular tanks supplied with running seawater. For *in vivo* dilution experiments, gradual dilution of the circulating water was accomplished by reducing salinity 10% per day down to 50% seawater. The skates were then allowed to equilibrate for two days prior to sampling. Fish were killed by swift transection of the spinal cord, and the beating heart was excised and placed in a beaker of balanced isotonic elasmobranch medium (Forster, R. P. et al., Comp. Biochem. Physiol. 42A:3-12, 1972) gassed with 99% O_2 - 1% CO_2 until clear of blood (1-2 minutes). The atrium was surgically separated from ventricle, the conus arteriosus was removed and pericardium trimmed off. For amino acid analyses, samples of atrium and ventricle were lightly blotted, weighed, placed in aluminum foil and frozen between blocks of dry ice. Tissue was ground to a powder by cold mortar and pestle on dry ice and extracted with 9 volumes of 4% sulfosalicylic acid as they thawed. The homogenates were centrifuged and the supernatants dried in a vacuum at 20°C over concentrated sulfuric acid. Amino acid analyses were performed on the dried residues with a Durrum D-500 automatic analyzer.

For the *in vitro* experiments, the excised heart was placed in gassed (99% O_2 - 1% CO_2) elasmobranch medium until clear of blood and then transferred to fresh medium. The atrium was surgically

removed and trimmed of pericardium and supporting structures. The separated thin walled atrium was divided in half and flattened to provide maximal surface area for diffusion. The hemi-atria were blotted, weighed and separate samples placed in Erlenmeyer flasks containing 1 ml medium for each 10 mg of tissue. Following preincubation for 15 minutes in the 99% O_2 - 1% CO_2 atmosphere at 15°C they were transferred to fresh medium containing 0.1 mmoles/l taurine and ^{14}C-labeled taurine. For the control uptake experiments atria were incubated for 15, 20, 30, 45, and 60 minutes. The specific activity of taurine in the medium was 2.2×10^8 dpm/mmole. Following incubation, the atrial samples were removed, rinsed in normal, nonradioactive medium, blotted lightly and placed in tared scintillation vials. The vials were weighed, 1 ml Protosol added to each, and the vials incubated overnight in a shaking water bath at 40°C. Upon removal from bath, 15 ml of scintillation cocktail (4 g PPO and .05 g POPOP in 1 l toluene) was added to each; the vials vortexed and assayed for ^{14}C by liquid scintillation counting.

To characterize the taurine transport system, tissue samples were incubated for one hour under a variety of experimental conditions following the 15 minute preincubation. In all cases, a paired analysis was performed with one hemi-atrium as control while the other was subjected to the experimental variable. For testing Na dependence, NaCl was completely omitted or partially replaced by choline chloride in the medium to give final Na concentrations of 0, 35, 70, 140 mM (control Na 280 mM). Energy dependence and competitive inhibition were tested by adding the following substances to the final incubation medium as required. N_3Na (10^{-3}M), ouabain (10^{-4}M), β-alanine (5×10^{-4}M), GABA (5×10^{-4}M) α-aminoisobutyric acid (5×10^{-4}M). Isoproterenol (4×10^{-7}M) and dibutyryl cyclic AMP (9.5×10^{-4}M) were added to the final incubation medium to test for possible effects of β-adrenergic stimulation on taurine uptake.

Results and Discussion

Table 1 shows the wet weight tissue contents of all detectable amino-containing compounds in skate ventricle and atrium from hearts of fish maintained in sea water and those gradually adapted to half-strength sea water. The major amino acid in both heart chambers is taurine, with concentrations averaging 84 and 66 μmoles/g wet wt respectively in ventricle and atrium of SW-adapted fish. These values are the highest reported to date in hearts of any species. Taurine is also the most common free amino acid in skate erythrocytes and brain but its concentration in skate striated muscle is far below that of sarcosine and β-alanine. The concentration in plasma averages 0.13 μmoles/ml and the taurine concentration in intracellular water of skate atrium is calculated as 139 μmoles/ml; so, close to a 1000-fold transmural gradient is normally maintained across myocardial cell membranes.

Table 1

Amino Compounds of Skate (*Raja erinacea*) Atria and Ventricles Taken From Fish Adapted to Full-strength and Half-strength Sea Water

	Ventricle		Atrium	
	SW	½ SW	SW	½ SW
Taurine	83.89 ± 7.12	80.7 ± 6.80	65.54 ± 4.57	58.5 ± 5.05
Hydroxyproline	0.23 ± 0.23	0.93 ± 0.20	-	-
Aspertic Acid	2.32 ± 0.64	1.03 ± 0.36	1.77 ± 0.43	1.29 ± 0.32
Threonine	0.53 ± 0.12	0.59 ± 0.12	0.37 ± 0.11	0.63 ± 0.10
Serine	0.45 ± 0.17	0.32 ± 0.07	0.20 ± 0.06	0.34 ± 0.07
Glutacine/Asparagine	-	-	-	-
Proline	-	-	-	-
Glutamic Acid	7.44 ± 0.81	5.60 ± 0.75	5.87 ± 0.59	5.49 ± 1.03
Citrulline	0.11 ± 0.08	-	-	-
Glycine	0.59 ± 0.21	0.48 ± 0.12	0.45 ± 0.10	0.49 ± 0.29
Alanine	0.46 ± 0.13	0.42 ± 0.14	0.46 ± 0.15	0.33 ± 0.17
Valine	0.35 ± 0.14	0.26 ± 0.03	0.11 ± 0.03	0.18 ± 0.01
Phosphoserine	0.62 ± 0.15	0.68 ± 0.21	0.61 ± 0.18	0.49 ± 0.13
Ethanolamine	5.19 - 1.15	6.15 ± 1.61	3.63 ± 0.97	4.27 ± 0.37
Phosphoethanolamine	0.54 ± 0.15	0.25 ± 0.32	1.11 ± 0.36	0.62 ± 0.01
Methionine	-	0.05 ± 0.02	-	-
Isoleucine	0.11 ± 0.04	0.02 ± 0.02	0.09 ± 0.02	0.14 ± 0.01
Leucine	0.23 ± 0.04	0.26 ± 0.06	0.17 ± 0.03	0.21 ± 0.05
Phenylalanine	-	0.06 ± 0.01	-	-
Ornithine	0.09 ± 0.06	0.04 ± 0.01	0.38 ± 0.01	0.03 ± 0.01
Lysine	0.03 ± 0.02	0.06 ± 0.02	-	0.08 ± 0.01
Histidine	0.07 ± 0.02	0.06 ± 0.03	0.02 ± 0.01	0.05 ± 0.01
Arginine	-	0.04 ± 0.02	-	0.05 ± 0.01
β-Alanine	-	-	-	-
Total	95.87 ± 6.43	98.90 ± 5.15	80.81 ± 5.99	73.50 ± 8.56

Values are means ± S.E. in μmoles/g wet wt tissue. SW, skates in seawater; ½ SW, skates adapted to half-strength seawater. n = 5.

Figure 1 represents net uptake of taurine by the semi-atrium *in vitro* preparation under previously described control conditions. Net uptake of taurine in 1 hr averages 1.43 μmoles/g wet wt of tissue from a control medium containing .1 μmole/ml taurine; i.e., net accumulation of new taurine added to a residual taurine content of 73 μmoles/g wet wt of tissue. Atrial tissue wet wt is 40% extracellular fluid which presumably also contains 0.1 μmole/ml taurine, similar to that of skate plasma.

A somewhat similar study of time-course taurine uptake by fetal mouse hearts showed that 6 μmole/g taurine accumulated during the first hour, or approximately 4x uptake rate in the skate. These Grosso et al. results (J. Clin. Invest. 61:944-952, 1978) were obtained at 37°C rather than 20°C, and their temperature-dependence studies showed that at 20°C the uptake rate was less than 1/2 that at 37°C. The intrinsically higher metabolic rate of hearts taken from fetuses of pregnant mice between 16 and 19 days of gestation probably is another factor accountable for their somewhat higher transport rate. We chose 1 hr as the standard incubation time for the subsequent studies on taurine uptake under various experimental conditions.

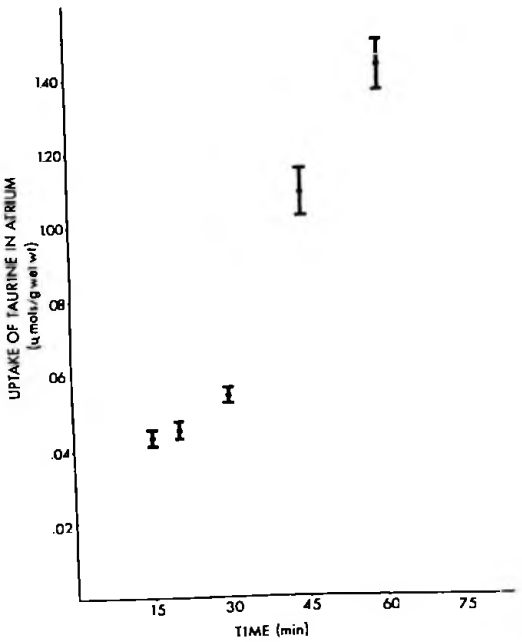

Figure 1. Time course of taurine uptake by skate atrium *in vitro*.

The dependence of taurine uptake upon sodium concentration was demonstrated with osmolality and chloride level held constant by the complete and step-wise replacement of NaCl with choline chloride (Table 2). Sodium dependence has previously been demonstrated for *in vitro* taurine transport into skate red blood cells and brain, as well as in many mammalian systems.

β-alanine, the carboxylic analogue of taurine, was an effective inhibitor of taurine transport. It decreased uptake 42% when present in 5x the taurine concentration. No significant effect was obtained with the simultaneous presence of α- and γ-structural analogues. Table 2 also shows the sensitivity of the taurine uptake system to the metabolic inhibitor, Na azide, and to the cardiotonic steroid, ouabain, in concentrations that inhibit the dephosphorylation reaction of Na^+-K^+ ATPase located on the outside face of the cell membrane.

One of the main reasons for our undertaking this study was to test the challenging hypothesis of Chubb and Huxtable (Science 198:409-411, 1977) that β-adrenergic activation triggers a series of events leading to stimulation of taurine transport by the heart. The absence of a sympathetic adrenergic nerve supply to the skate's heart, as is the case with all elasmobranchs, would predict that perhaps a β-adrenergic agonist such as isoproterenol, and an intracellular mediater of its effects such as dibutyryl cyclic AMP, would be ineffective here in stimulating taurine transport. In the rat heart, with its usual adrenergic innervation, they were both found to be effective stimulatory agents. Our experiments on the skate showed this indeed to be true; isoproterenol and cyclic AMP had no effect on taurine transport. However, the significance of this comparative experiment was blunted by the finding that whereas β-adrenergic stimulation does not increase taurine transport by the skate heart, isoproterenol and other catecholamines do have a positive inotropic effect on skate atrial and ventricular myocardium, despite the absence of an adrenergic nerve supply to the heart.

The results of these studies are presented separately in this issue of the Bulletin (Forster, Hannafin, Shiffrin and Morad).

Conclusions

Uptake of ^{14}C taurine occurs *in vitro* against a strong concentration gradient when skate "hemi-atria" are incubated in a balanced isosmotic elasmobranch medium containing 0.1 mM taurine. The transport system is Na-dependent, and presumably carrier-mediated. The close structural analogue, β-alanine, reduces taurine uptake by 42% when present in 5-fold excess. α-aminoisobutyric acid, and γ-aminobutyric acid (GABA) do not inhibit uptake. The system is energy-dependent and subject to Na^+-K^+ ATPase inhibition by ouabain. No evidence was found to indicate adrenergic stimulation of taurine transport, as has previously been shown in experiments on mammalian hearts. This work was supported by NIH Grant HL 04457-20.

Table 2

Taurine Uptake by Skate Atrium, Expressed as Percent of Control, as Affected by Various Agents Selected to Characterize the Transport System

Treatment	Percent decrease in uptake of taurine	
Na dependence		
0 mM Na	-76.22 ± 3.71%	(4)**
35 mM Na	-56.27 ± 10.3%	(6)**
70 mM Na	-49.71 ± 7.76%	(6)**
140 mM Na	-30.35 ± 12.48%	(6)**
Competitive inhibition		
β-Alanine (10^{-5}M)	-42.40 ± 6.50%	(11)*
γ-Aminobutyric acid (10^{-5}M)	n.s.	(9)
α-Aminoisobutyric acid (10^{-5}M)	n.s.	(7)
Metabolic dependence		
Na azide (10^{-2}M)	-58.74 ± 1.27%	(3)*
Na^+-K^+ ATPase inhibition		
Ouabain (10^{-4}M)	-44.76 ± 2.89%	(4)*
β-adrenergic effect		
Isoproterenol (4×10^{-7}M)	n.s.	(7)
Dibutyryl cyclic AMP (9.5×10^{-4}M)	n.s.	(6)

Value are means ± standard deviation. Number of fish per group is shown in parentheses. *$p < 0.001$, **$p < 0.01$, n.s. = not significantly different from control.

EFFECTS OF UREA AND HIGH P_{O_2} ON ACID SECRETION IN DOGFISH STOMACH

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The dogfish gastric mucosa, as mounted in an Ussing-type chamber and gassed with 95% O_2 - 5% CO_2, is both hypoxic and hypocapnic, and increasing P_{O_2} to 1.9 atm and P_{CO_2} to 0.1 atm by a hyperbaric apparatus markedly increases secretory rate (Kidder, G. W., Bull. MDIBL 15:68, 1975). Since Hogben (Science 129:1224, 1959) has reported that in 95% O_2 - 5% CO_2 the inclusion of urea in the bathing solutions had no effect on the secretory parameters, urea was not used in the initial hyperbaric experiments. However, with the removal of O_2-limitation, it seemed desirable to reinvestigate this observation.

Accordingly, the gastric mucosa of freshly-killed dogfish was dissected free of the heavy muscle coat and mounted in a plexiglass chamber (3.14 cm^2 area). The serosal surface was bathed with the solution used by Hogben, containing (mM) Na^+, 239; K^+, 10; Ca^{++}, 5; Mg^{++}, 2; Cl^-, 244; HCO_3^-, 18; phosphate, 0.6 and glucose, 25. The mucosal solution was similar, but without glucose, phosphate or HCO_3^-, the latter two being replaced by NaCl. Urea-containing solutions were prepared by adding 350 mM urea to each of the above. All tissues were stimulated by carbachol at 0.25 mM on the serosal surface.

Figure 1 shows the experimental results for one tissue. This tissue was mounted in urea-containing solutions and allowed to equilibrate for 1.5 hours at P_{O_2} = 1.9 atm. The pressure was then released, the solutions changed to their urea-free equivalents, and the pressure reestablished, a process which takes about 10 minutes. After 1.5 hours in this condition, the tissue was returned to urea-containing solutions for 1.5 hours. In this particular experiment (but not generally) an additional 1.5 hours in urea was allowed. It seems clear that the secretory rate has decreased upon removal of the urea, and increases upon readdition of urea, although not to its initial level. In this experiment it would have been desirable to allow longer than 1.5 hours for initial equilibration; failure to do so tends to bias the results against finding any effect of urea.