Taurine: Possible Role in Osmotic Regulation of Mammalian Heart

Abstract. *It is well established that taurine plays an important role in the maintenance of intracellular osmolal concentration in marine invertebrates, teleosts, and amphibians. In fresh water, concentrations of taurine in body tissues decrease; in salt water, they increase. In this study with mice we found that during adaptation of these mammals to chronic hypernatremia, the taurine content of the heart increased; concentrations of other amino acids were unchanged or were decreased. Welty and his associates have shown that acute hyponatremia lowered the taurine concentration of rat heart. In concert, these data suggest that taurine also may serve as an osmotic agent in mammalian heart.*

The concentration of taurine in mammalian heart is high, amounting to about 60 percent of the total free amino acid pool (*1*). The physiologic function of taurine in the heart is not clear (*2*, *3*). In lower animal species, taurine plays an important role in the maintenance of osmotic equilibrium across cell membranes. A few examples are of interest. Freshwater and terrestrial mollusks contain little or no taurine, but all marine forms do, as high as 70 mmole per kilogram of fresh weight in some species (*4*). During hypoosmotic stress the taurine content of isolated crab axons decreased 37 percent; under hyperosmotic stress, the taurine concentration increased 24 percent (*4*). Amphibia exhibit a similar response: during adaptation to increasing environmental salt concentrations the taurine content of skeletal muscle increased from 47 to 164 percent (*5*). In flounders adapted to fresh water, the taurine content of heart muscle decreased 40 percent (*5a*).

We recently reported data supporting a role for taurine in osmotic regulation in mammalian brain (*6*). In chronically hypernatremic mice (mean plasma sodium concentration 186 meq/liter), 16 of 19 brain amino acids were significantly increased. On a molar basis, the largest increases were in asparate, glutamate, glutamine, γ-aminobutyric acid, glycine, serine, and taurine. The sum of the increases of these seven amino acids above the control value was 53 mmole per kilogram of dry weight. Taurine accounted for over one-half of this increment (30 mmole/kg). By comparison, the sodium content of the brains of these animals increased by 29 meq per kilogram of dry weight and potassium by 17 meq/kg (*6*).

Data reported by Welty *et al.* (*7*) suggested that taurine might also function as an osmoregulator in mammalian heart. When plasma sodium was lowered to 102 meq/liter over a 4-hour period by intraperitoneal injection of a 5.5 percent glucose solution, rat heart taurine was reduced more than one-third.

To test the hypothesis that taurine was an osmotic agent in mammalian heart, we used young mice with chronic hypernatremia (*6*). After 4 days of water deprivation and daily injections of a molar sodium chloride solution, the experimental animals weighed 36 percent less than controls (Table 1), but showed no differences in either appearance or clinical behavior.

In salt-loaded mice, plasma sodium concentration increased to 185 meq/liter (Table 1). In the hearts of these animals, taurine increased 30 percent on a wet weight basis and 16 percent on a dry weight basis (Table 1). Chronic hypernatremia did not increase the other amino acids in the heart. In fact, on a dry weight basis there were significant decreases in cardiac levels of aspartate (19 percent), glutamate (16 percent), and glycine (12 percent). In other tissues, salt-induced hyperosmolality was associated with significant increases in the levels of these and other amino acids (as

Table 1. The effect of chronic hypernatremic dehydration on body weight and concentrations of selected substances in the hearts and plasma of young mice. Normal nursing (17 to 23 days old) mice were removed from their mothers and deprived of water for 4 days. During this interval they were given free access to dry food and received subcutaneous injections of molar NaCl (20 ml/kg) once or twice daily (total, seven injections). Control littermates received an equivalent volume of 0.9 percent NaCl and were given free access to food and water. Two hours after the last injection the mice were decapitated and blood was collected from the severed neck vessels in heparinized capillary tubes. The capillary tubes were centrifuged at 4°C and extracts of the plasma were prepared in perchloric acid (*13*). For water and electrolyte measurements, heart was dissected at room temperature. Methods for determination of heart water, and heart and plasma electrolyte concentrations are described in (*14*). After decapitation the heart was quickly removed at room temperature; blood was expressed by gentle pressure with tissue paper and the heart was dropped into liquid nitrogen. Frozen hearts were dissected free of blood and membranes in a cryostat at − 35°C. Tissue extracts were prepared by the method of Lowry and Passonneau (*13*). Taurine was measured by the fluorescamine method of Orr *et al.* (*15*). Alanine, glutamate, and aspartate were measured by the methods of Lowry and Passonneau (*13*), glutamine by the method of Young and Lowry (*16*), and γ-aminobutyrate by a minor modification of the method of Hirsch and Robins (*17*). The method of glycine assay was that of Berger *et al.* (*18*). Heart amino acid concentrations are expressed in millimoles per kilogram of wet weight (ww) and in millimoles per kilogram of dry weight (dw). Each value is the mean ± standard error for 6 to 11 mice; N.S., not significant, $P > .05$.

Measurement	Control	Hypernatremia	P
	Body weight		
Initial (g)	12.2 ± 0.5	12.0 ± 0.5	N.S.
Final (g)	16.2 ± 0.6	10.3 ± 0.5	< .001
	Plasma electrolytes		
Na^+ (meq/liter)	147 ± 1.0	185 ± 4.0	< .001
K^+ (meq/liter)	7.8 ± 0.4	7.5 ± 0.2	N.S.
	Plasma amino acids		
Taurine (mmole/liter)	0.65 ± 0.06	0.77 ± 0.10	N.S.
	Heart water and electrolytes		
Water (g/100 g, ww)	75.57 ± 0.20	72.42 ± 0.26	< .001
Water (g/100 g, dw)	310 ± 3.0	263 ± 3.0	< .001
Na^+ (meq/kg, ww)	41.0 ± 0.7	49.9 ± 1.4	< .001
Na^+ (meq/kg, dw)	168 ± 3.0	181 ± 5.0	.05
K^+ (meq/kg, ww)	88.9 ± 1.0	104 ± 2.0	< .001
K^+ (meq/kg, dw)	364 ± 4.0	379 ± 8.0	N.S.
	Heart amino acids		
Taurine (ww)	33.9 ± 1.1	44.2 ± 0.7	< .001
Taurine (dw)	139 ± 4.0	160 ± 4.0	.001
Aspartate (ww)	2.87 ± 0.14	2.68 ± 0.15	N.S.
Aspartate (dw)	11.8 ± 0.6	9.6 ± 0.54	.017
Alanine (ww)	0.838 ± 0.028	0.915 ± 0.042	N.S.
Alanine (dw)	3.29 ± 0.12	3.36 ± 0.14	N.S.
Glycine (ww)	1.63 ± 0.05	1.76 ± 0.09	N.S.
Glycine (dw)	7.00 ± 0.16	6.13 ± 0.22	.004
Glutamate (ww)	6.18 ± 0.19	5.82 ± 0.29	N.S.
Glutamate (dw)	24.4 ± 0.5	20.4 ± 0.7	< .001
Glutamine (ww)	5.63 ± 0.28	6.56 ± 0.36	N.S.
Glutamine (dw)	24.2 ± 0.7	27.7 ± 1.9	N.S.
γ-Aminobutyrate (ww)	0.523 ± 0.029	0.507 ± 0.067	N.S.
γ-Aminobutyrate (dw)	2.14 ± 0.12	1.99 ± 0.25	N.S.

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well as taurine) (*5*, *6*). We have no explanation for this difference.

The mechanism of the increased taurine content in the hearts of the hyperosmotically stressed mice is unknown. The heart can both synthesize taurine and transport taurine from the circulation (*1*). Since the uptake of taurine into myocardial membranes is sodium-dependent (*8*), the latter mechanism may operate in hypernatremic mice.

Whether taurine has a function in heart is not known (*2*, *3*, *9*). Although we cannot rule out other possible explanations (*2*, *3*, *10–12*) the striking decrease in heart taurine during hypoosmotic stress (*2*, *3*) and increase during hyperosmotic stress support the possibility that taurine acts as an osmotic agent in mammalian heart.

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Hydrogen Isotope Ratios of Mouse Tissues Are Influenced by a Variety of Factors Other Than Diet

In a recent report, Estep and Dabrowski (*1*) presented data which they claimed defined the relationship between the stable hydrogen isotopic ratios of an animal's tissues and those of its food and water sources. On the basis of their interpretation of these data, they suggested that the isotopic composition of animal hydrogen records information about an animal's diet. We wish to comment on their report.

Estep and Dabrowski (*1*) studied a single group of mice that had eaten a diet of a fixed ratio of deuterium to hydrogen (D/H) and drunk water whose D/H ratio differed from that of the diet by a constant amount. They found that the D/H ratios of dried samples of liver, muscle, and feces were similar to the D/H ratio of the food and dissimilar to the D/H ratio of the water. They concluded that the hydrogen isotopic composition of the food an animal eats rather than that of the water it drinks controls the isotopic composition of the organically bonded hydrogen in its tissues.

Table 1. Stable hydrogen isotopic ratios of mouse food, mouse tissues, and cellulose nitrate that were exposed to water vapors of different isotopic composition. Food and tissue samples from 11 BALB/c female mice raised on Wayne Lab-Blox F6 mouse food and tap water (*8*) were pooled, homogenized, and freeze-dried. Aliquots of dried food, liver, and brain were held for 1 week in two cylinders above liquid water whose δD values are given. Aliquots of dried cellulose nitrate prepared from mangrove wood (*3*) were exposed to the same conditions for 2 weeks. All samples were vacuum-dried for 24 hours prior to conversion of their organically bonded hydrogen to water by the Stump and Frazer combustion procedure (*9*), reduction of the water to hydrogen gas by the procedure of Bigeleisen *et al.* (*10*), and determination of the isotopic ratios of the hydrogen gas by mass spectrometry. The D/H ratios are given as δD values (*6*). The precision of the δD determinations was ± 2 per mil.

Sample	δD_{SMOW} (per mil)		
	Experiment 1	Experiment 2	Experiment 2 minus experiment 1
Water	−79	+128	+207
Brain	−146	−112	+34
Liver	−81	−30	+51
Food	−55	+5	+60
Cellulose nitrate	+16	+17	+1

Hydrogen isotopes are fractionated during biochemical reactions in a variety of organisms (*2–5*). Indeed, Estep and Hoering (*2*) observed an isotopic fractionation between some plants and the water in which they grew that was about as large as the difference Estep and Dabrowski (*1*) reported between the D/H ratios of the mouse tissues and the water the mice drank. It is certainly possible that the fractionations that occur during the incorporation of hydrogen from water into the proteins, lipids, and other compounds of which organisms are comprised are of the same sense and magnitude in both plants and animals. The data reported by Estep and Dabrowski (*1*) do not eliminate this possibility. Several types of experiments could be done to determine whether the D/H ratios of food control the D/H ratios of animals. One could analyze tissues from animals raised on food and water whose D/H ratios differ so drastically (by a factor of 50 or more) that any reasonable fractionations could not interfere with interpretation of the results. One might also analyze tissues from at least two sets of mice raised on food of the same isotopic composition but supplied with waters whose D/H ratios differed by a large amount.

The experiments we suggest above probably will show that the D/H ratios of animals and their tissues are not controlled solely by the D/H ratios of their food. We have done a simple experiment which indicates that the D/H ratios of a significant fraction of the organically bonded hydrogen in animal tissues must be determined by the isotopic composition of water that the samples encounter. We exposed aliquots of dried mouse brain and liver and mouse food to water vapors of different D/H ratios prior to isotopic analysis. The data in Table 1 indicate that at least 16 percent of the hydrogen in mouse brain is exchangeable with the hydrogen of water; the corresponding values for mouse liver and mouse food are 25 and 29 percent. By contrast, none of the hydrogen in cellulose nitrate, a sample we analyzed as a control since its hydrogen is bonded nonexchangeably to carbon (*3*), exchanges under these conditions (Table 1). These values represent minimum estimates of the amount of exchangeable hydrogen in the different samples, because we made no effort to determine if the exchange process had gone to completion. Ex-

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