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### Research Article

Cardiac taurine levels are elevated in hypertension and congestive heart failure. A possible mechanism for this increase in taurine is an alteration of its uptake. We sought to identify and characterize a carrier-mediated transport system for taurine in the mammalian myocardium utilizing the fetal mouse heart in organ culture. Hearts from fetuses of 16-19 days gestational age used in these studies had an endogenous taurine content of  $14.1 \pm 0.5$  nmol/mg tissue.

The uptake of [ $^3$ H]taurine was linear for up to 8 h. Taurine was accumulated against a concentration gradient as demonstrated by a net increase in taurine concentration when hearts were incubated in 0.5 mM taurine. [ $^3$ H]Taurine uptake was saturable,  $K_m = 0.44$  mM, temperature dependent, and required sodium. The close structural analogues, hypotaurine and  $\beta$ -alanine, reduced [ $^3$ H]taurine uptake by 87% when present in 100-fold excess. The  $\alpha$ -amino acids alanine,  $\alpha$ -aminoisobutyric acid, glycine, leucine, and threonine did not inhibit uptake. Other taurine analogues tested were guanidinotaurine, guanidinopropionic acid,  $\gamma$ -aminobutyric acid, 2-aminoethane phosphonic acid, aminomethane sulfonic acid, 3-aminopropane sulfonic acid, *N*-acetyltaurine, and isethionic acid. We conclude that a carrier-mediated transport system for taurine exists in the fetal mouse heart based on the demonstration of (a) temperature dependence, (b) saturability, and (c) structural selectivity of the uptake process. Transport was demonstrated to be mediated by a  $\beta$ -amino acid uptake system. In addition, taurine [...]

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# Characterization of a Carrier-Mediated Transport System for Taurine in the Fetal Mouse Heart In Vitro

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**ABSTRACT** Cardiac taurine levels are elevated in hypertension and congestive heart failure. A possible mechanism for this increase in taurine is an alteration of its uptake. We sought to identify and characterize a carrier-mediated transport system for taurine in the mammalian myocardium utilizing the fetal mouse heart in organ culture. Hearts from fetuses of 16–19 days gestational age used in these studies had an endogenous taurine content of  $14.1 \pm 0.5$  nmol/mg tissue.

The uptake of [ $^3\text{H}$ ]taurine was linear for up to 8 h. Taurine was accumulated against a concentration gradient as demonstrated by a net increase in taurine concentration when hearts were incubated in 0.5 mM taurine. [ $^3\text{H}$ ]Taurine uptake was saturable,  $K_m = 0.44$  mM, temperature dependent, and required sodium. The close structural analogues, hypotaurine and  $\beta$ -alanine, reduced [ $^3\text{H}$ ]taurine uptake by 87% when present in 100-fold excess. The  $\alpha$ -amino acids alanine,  $\alpha$ -aminoisobutyric acid, glycine, leucine, and threonine did not inhibit uptake. Other taurine analogues tested were guanidinotaurine, guanidinopropionic acid,  $\gamma$ -aminobutyric acid, 2-aminoethane phosphonic acid, aminomethane sulfonic acid, 3-aminopropane sulfonic acid, *N*-acetyltaurine, and isethionic acid. We conclude that a carrier-mediated transport system for taurine exists in the fetal mouse heart based on the demonstration of (a) temperature dependence, (b) saturability, and (c) structural selectivity of the uptake process. Transport was demonstrated to be mediated by a  $\beta$ -amino acid uptake system. In addition, taurine uptake was observed to be sodium dependent, energy dependent, and capable of accumulating taurine against a concentration gradient.

## INTRODUCTION

Taurine (2-aminoethane sulfonic acid) is present in high concentrations as the free amino acid in the

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normal myocardium (1–3). Interest in the role of taurine in cardiac physiology derives from the observation of its ability to mitigate the toxic effects of the digitalis glycosides (4, 5). Myocardial taurine levels have been found to be elevated in patients with hypertension and patients who died of heart failure (6, 7). Similar observations have been made in animal models of cardiac hypertrophy (3, 8) and hypertension (7). Electrophysiological (5, 9) and biochemical (10) studies have reported that taurine alters ion permeabilities in the heart.

Two important mechanisms that could account for the high levels of taurine in the heart are biosynthesis and uptake. The known pathways for taurine synthesis in liver and brain are absent from the heart (11). *In vivo* studies have demonstrated a slow accumulation of taurine from the circulation by the heart (12, 13). Preliminary reports utilizing *in vitro* models indicate that a taurine transport system exists in the mammalian heart (14–16).

The intact, beating, fetal mouse heart in organ culture provides a useful *in vitro* model for the study of myocardial metabolism (17, 18). With 8–16 fetuses per litter, sufficient numbers of hearts are obtained to allow direct comparison between experimentally treated hearts and matched littermate control hearts, thereby minimizing individual variability. In addition, this model permits the systematic manipulation of experimental conditions which would be difficult to achieve using large animal models or perfused hearts.

Thus, we chose the fetal mouse heart to demonstrate that the uptake of taurine into the mammalian myocardium satisfies the generally accepted criteria of a carrier-mediated process: (a) temperature dependence, (b) saturability, and (c) structural specificity. In addition, we characterized the dependence of this system on sodium ion and energy and demonstrated that it was distinct from the known transport systems for neutral  $\alpha$ -amino acids.

## METHODS

**Organ culture conditions.** Hearts were removed aseptically from fetuses of pregnant CFI strain white mice (Charles River Breeding Laboratories, Wilmington, Mass.) between 16 and 19 days of gestation and placed in culture as previously described (19, 20). The hearts, 2–4 mg wet wt, were dissected free from pericardium and vessels and placed on stainless steel grids in organ culture dishes (Falcon Plastics, Div. BioQuest, Oxnard, Calif.) containing medium 199 (Microbiological Associates Inc., Bethesda, Md.) with Earle's salts (Grand Island Biological Co., Grand Island, N. Y.). The hearts were kept in airtight culture jars under an atmosphere of 95% O<sub>2</sub>/5% CO<sub>2</sub> at 37°C.

**Uptake and efflux.** The uptake of taurine was measured in hearts from matched littermates. The hearts were incubated individually in culture dishes for 1 h at 37°C under an atmosphere of 95% O<sub>2</sub>/5% CO<sub>2</sub> after which they were observed for beating. They were randomly divided into control and experimental groups and then transferred to new culture dishes containing a measured volume of medium 199 supplemented with 20  $\mu$ Ci/ml [<sup>3</sup>H]taurine and 1.5  $\mu$ Ci/ml [<sup>14</sup>C]sorbitol. After 1 h of incubation the hearts were removed, blotted, and weighed. They were dissolved by heating for 1 h at 60°C in 0.3 ml of 0.3 N NaOH. The solution was then neutralized with 0.3 N HCl. Radioactivity was measured by liquid scintillation counting in a Beckman model 250 liquid scintillation spectrometer (Beckman Instruments, Inc., Spinco Div., Fullerton, Calif.) equipped with AQC using a toluene-based fluor containing 30% (vol/vol) Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) and 4 g/liter 2,5-diphenyloxazole (New England Nuclear, Boston, Mass.).

The efflux of taurine was measured in hearts that were first incubated individually for 2 h in 0.01 mM [<sup>3</sup>H]taurine (20  $\mu$ Ci/ml) and [<sup>14</sup>C]sorbitol (1.5  $\mu$ Ci/ml). The hearts were then rinsed and placed in fresh medium containing either no taurine or 1.0 mM unlabeled taurine. At selected times, the medium was removed for counting, replaced by fresh medium, and the incubation continued. The total [<sup>3</sup>H]taurine content of each heart was the sum of the [<sup>3</sup>H]taurine that appeared in the medium at each time period and the [<sup>3</sup>H]taurine remaining in the heart at the end of the 8 h of incubation. These values were then normalized.

**Taurine analysis.** Taurine was measured with the aid of an amino acid analyzer (15). The retention time for taurine was 26 min. This method separates taurine from other acidic amino acids such as phosphoethanolamine, cysteic acid, and cysteine sulfonic acid.

**Net [<sup>3</sup>H]taurine uptake.** The nontransportable carbohydrate [<sup>14</sup>C]sorbitol was used to estimate the extracellular space within each heart (21). The total [<sup>3</sup>H]taurine measured in each heart was corrected for the sorbitol space to yield the net [<sup>3</sup>H]taurine taken up into the hearts. Total water content of the hearts was determined by difference between wet and dry weights. Intracellular water was obtained by difference between total water and sorbitol space.

**Statistics.** All statistical analyses were performed by computer, CDC 6400, using the Statistical Procedures for the Social Sciences (22) software package. Comparisons between treatment means and control were made by Student's *t* test. Comparisons among all treatment means within an experiment were made with Duncan's New Multiple-Range test. Best-fit lines were determined by a least-squares linear regression method or a least-squares nonlinear regression analysis. To determine whether the slopes of two regression lines were significantly different from one another, an analysis of covariance was performed by a stepwise, dummy variable, multiple regression procedure.

**Chemicals and special media.** Taurine was from Sigma Chemical Company. [<sup>3</sup>H]Taurine, 3.9 Ci/mmol, was custom synthesized by catalytic exchange by New England Nuclear. It was purified by ion-exchange chromatography in acetone/formic acid/water (16/3/9, vol/vol/vol). It was determined to be 98%+ pure by thin layer chromatography and by isotopic dilution and recrystallization to constant specific activity. D[U-<sup>14</sup>C]Sorbitol, 100 mCi/mmol, was obtained from New England Nuclear.

Glucose-free medium and sodium-free medium were made from appropriate inorganic salts and the component solutions supplied by Grand Island Biological Co.: Eagle's Minimal Essential Medium (MEM)<sup>1</sup>-vitamin<sup>1</sup> solution ( $\times 100$ ), antibiotic-antimycotic mixture ( $\times 100$ ), MEM-amino acid solution ( $\times 50$ ), and L-glutamine (200 mM). Oxygen-free medium was prepared by removing dissolved gases with a vacuum pump and replacing the atmosphere with nitrogen.

The following substances were added to the medium as required:  $\beta$ -alanine, 2-aminoethane phosphonic acid and hypotaurine (Calbiochem, San Diego, Calif.); taurine,  $\alpha$ -L-alanine,  $\gamma$ -aminobutyric acid,  $\alpha$ -aminoisobutyric acid, 2,4-dinitrophenol, glycine, guanidinopropionic acid, isethionic acid,  $\alpha$ -L-leucine,  $\alpha$ -L-threonine, and ouabain (Sigma Chemical Co.); 3-aminopropane sulfonic acid (Chemical Procurement Laboratories, College Point, N. Y.); aminomethanesulfonic acid (Aldrich Chemical Co., Inc., Milwaukee, Wis.); guanidino-*t*aurine, synthesized according to a previously reported method (23). Guanidino-*t*aurine had a melting point of 272–273°C, and elemental analysis gave C, 21.56; H, 5.34; N, 25.15; S, 19.16%. *N*-Acetyltaurine was prepared by reacting taurine with acetic anhydride in pyridine at 60°C overnight. The *N*-acetyltaurine was chromatographically homogeneous, contained no taurine by analysis on an amino acid analyzer, and exhibited a nuclear magnetic resonance spectrum consistent with *N*-acetyltaurine.

## RESULTS

**Taurine content.** Fetal mouse hearts weighing from 2–4 mg (wet wt) which contained  $14.1 \pm 0.5$  nmol (mean  $\pm$  SEM) of taurine/mg of tissue ( $n = 23$ ) were used in the experiments described below. Hearts randomly chosen from seven separate litters were used for the taurine determinations. Total water content of the hearts was  $0.785 \pm 0.009$   $\mu$ l/mg tissue and sorbitol space was  $0.208 \pm 0.008$   $\mu$ l/mg tissue. Assuming a single intracellular pool within the heart, as previously demonstrated in the brain (24), the concentration of taurine is then calculated to be approximately 25 mM.

**Time-course.** Taurine uptake over a 4-h period in fetal mouse hearts is shown in Fig. 1. Hearts were incubated individually for selected times between 15 min and 4 h in medium 199 supplemented by 5  $\mu$ M or 2 mM [<sup>3</sup>H]taurine. Additional experiments not shown also demonstrated that the uptake was linear at 0.2 and 1.0 mM over the same 4-h period and was linear up to 8 h at 0.5 mM taurine. Accordingly, we chose 1 h as the standard time for incubation of hearts for the subsequent study of [<sup>3</sup>H]taurine uptake under various experimental conditions.

<sup>1</sup>Abbreviation used in this paper: MEM, Eagle's Minimal Essential Medium.

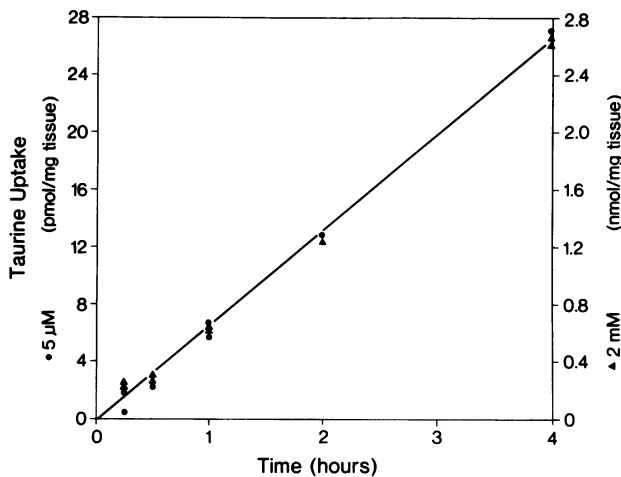


FIGURE 1 Time-course of taurine uptake. Individual hearts were incubated for  $\frac{1}{4}$ ,  $\frac{1}{2}$ , 1, 2, or 4 h at  $37^{\circ}\text{C}$  in medium 199 containing  $5\ \mu\text{M}$  (●) or 2 mM (▲) [ $^3\text{H}$ ]taurine. Each point represents the net taurine uptake into a single heart.

In a separate experiment, a net accumulation of taurine was observed after incubation for 4 h in medium containing 0.5 mM taurine. Hearts from three separate litters were divided equally into two groups of 16 hearts each. One group of hearts was incubated in taurine-free medium and one group was incubated in taurine-containing medium. Control hearts contained  $14.2 \pm 0.7$  nmol taurine/mg wet wt (mean  $\pm$  SEM), and hearts incubated with taurine contained  $16.3 \pm 0.6$

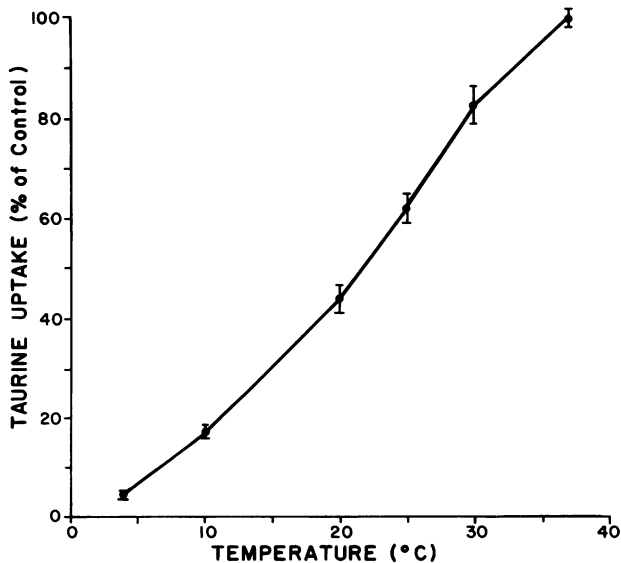


FIGURE 2 Effect of temperature on taurine uptake. Hearts were incubated in medium 199 containing 0.2 mM taurine at  $4^{\circ}$ ,  $10^{\circ}$ ,  $20^{\circ}$ ,  $25^{\circ}$ ,  $30^{\circ}$ , and  $37^{\circ}\text{C}$  for 1 h. A total of 27 hearts from three litters were used. The data are presented as means  $\pm$  SEM of the percentage of the uptake observed at  $37^{\circ}\text{C}$ .

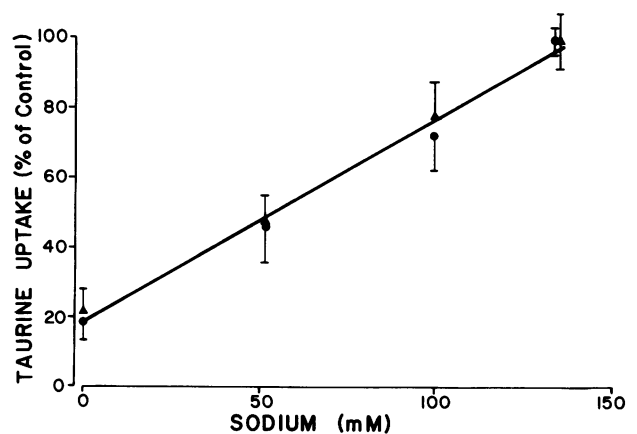


FIGURE 3 Effect of removal of sodium on taurine uptake. Hearts were incubated with 0.2 mM [ $^3\text{H}$ ]taurine for 1 h at  $37^{\circ}\text{C}$  in Tris phosphate-buffered MEM at a constant osmolality of 300 mosM. Sodium chloride was replaced with sucrose (●) or lithium chloride (▲). A total of 54 hearts from six litters were used. The data are presented as means  $\pm$  SEM for the uptake as a percentage of the uptake at 135 mM sodium chloride.

nmol/mg wet wt. The difference was statistically significant,  $P < 0.05$ .

**Temperature dependence.** The rate at which the hearts accumulated taurine from the medium was temperature dependent as seen in Fig. 2. An energy of activation of 7.4 kcal/mol, equivalent to a  $Q_{10}$  of 1.5, was calculated for the temperature range of  $30$ – $37^{\circ}\text{C}$ .

**Sodium dependence.** Taurine uptake was observed to be dependent upon the sodium concentration of the medium as shown in Fig. 3. In one set of three experiments osmolality was held constant by replacing sodium chloride with sucrose. In a second set of three experiments, both ionic strength and osmolality were maintained by replacement of the sodium chloride with lithium chloride. This offered the added advantage of maintaining a constant chloride concentration. The complete substitution of sodium chloride with sucrose or lithium chloride reduced the uptake of taurine an equivalent amount.

**Concentration dependence.** The dependence of the rate of uptake on the concentration of taurine in the medium is demonstrated in Fig. 4. Taurine uptake was measured over the concentration range of 0.05 to 1.8 mM in the presence and in the absence of sodium. For studies requiring sodium-free medium, either sucrose or lithium chloride was substituted for sodium to maintain the osmolality of the medium at the same level as the sodium-containing medium. The experiments with lithium chloride also maintained the ionic strength and chloride concentration at the levels of the medium containing sodium. Taurine uptake was the same in both sets of three experiments. For this

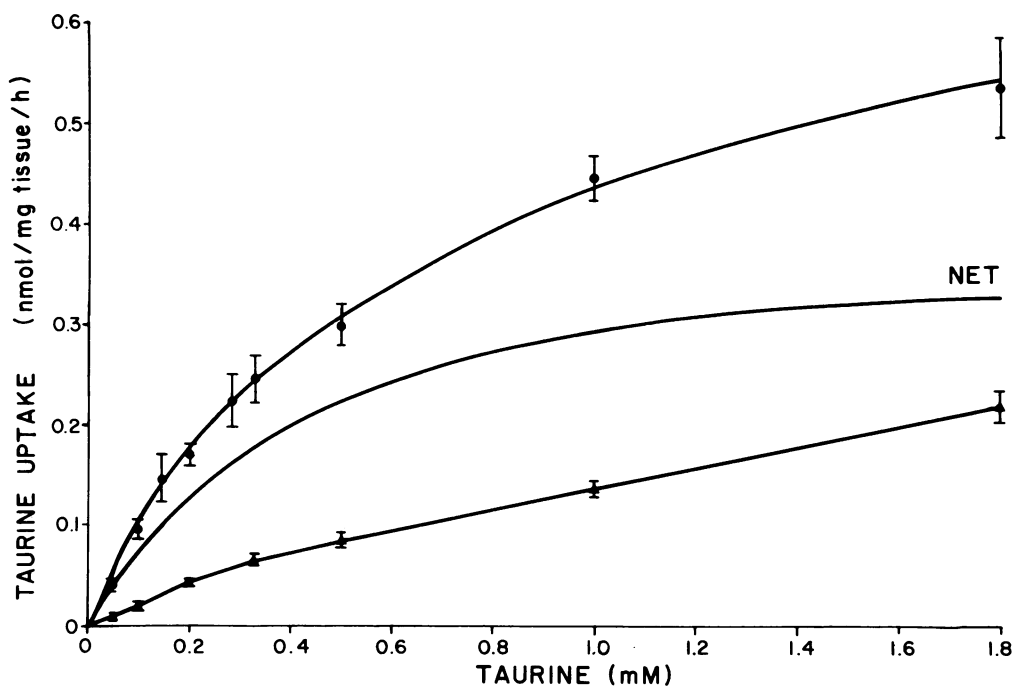


FIGURE 4 Concentration dependence of the rate of taurine uptake. Hearts were incubated in Tris phosphate-buffered MEM containing 0.05–1.8 mM [ $^3\text{H}$ ]taurine for 1 h at 37°C. Uptake was measured in the presence (●) and absence (▲) of sodium at a constant osmolarity of 300 mosM. Net = the difference between [ $^3\text{H}$ ]taurine uptake in the presence and absence of sodium. A total of 108 hearts from nine litters were used. The data are presented as means  $\pm$  SEM.

reason, the data were combined to obtain Fig. 4. The net sodium-dependent taurine uptake, obtained by subtracting the uptake in sodium-free medium from that at the normal sodium concentration, into the fetal mouse heart is a saturable process.

Estimates of the kinetic parameters for the net sodium-dependent uptake of taurine were obtained by two separate methods from the data shown in Fig. 4. A least-squares linear regression analysis of a double-reciprocal plot of the data as shown in Fig. 5 gave a value for  $K_m$  of 0.44 mM and  $V_{max}$  of 0.40 nmol/mg tissue per h. A nonlinear regression analysis (22) using a computerized procedure for obtaining the best-fit line to the actual data for sodium-dependent taurine uptake gave a value for  $K_m$  of 0.43 mM and for  $V_{max}$  of 0.41 nmol/mg tissue per h.

**Countertransport.** Carrier-mediated transport systems for organic substances generally are capable of transporting substrate in both directions across the cell membrane. This phenomenon is referred to as countertransport (25) and is characterized by a greater rate of efflux of substrate from the intracellular pool when the bathing medium contains substrate than when the medium is substrate-free. Fig. 6 represents measurements made of the efflux of [ $^3\text{H}$ ]taurine from hearts preloaded for 2 h. After the preincubation

period, one group of hearts was incubated in taurine-free medium and a second group in 1.0 mM unlabeled taurine.

Two components of efflux are evident in Fig. 6. The rapid efflux of [ $^3\text{H}$ ]taurine from the hearts during the early phase paralleled the efflux of [ $^{14}\text{C}$ ]sorbitol from the hearts reflecting clearance of the extracellular space. The remaining portion of the curve represents efflux from the intracellular space. The rate of efflux of [ $^3\text{H}$ ]taurine from the intracellular space was greater from the hearts incubated in the presence of taurine than from the hearts incubated in the absence of taurine. The slopes of the two curves were significantly different,  $P < 0.001$ , as determined by multiple regression analysis. Statistically significant differences were also observed between the taurine efflux from the two groups of hearts at 4 h,  $P < 0.05$ , and 8 h,  $P < 0.01$ . These data are consistent with countertransport and provide additional evidence that taurine accumulation is a carrier-mediated process.

**Energy dependence.** Data presented in Table I demonstrate that between 70 and 80% of the taurine uptake into the fetal mouse heart is energy dependent. The accumulation of [ $^3\text{H}$ ]taurine by hearts incubated under nitrogen was reduced by 72% from that in hearts incubated with oxygen,  $P < 0.001$ . Glucose

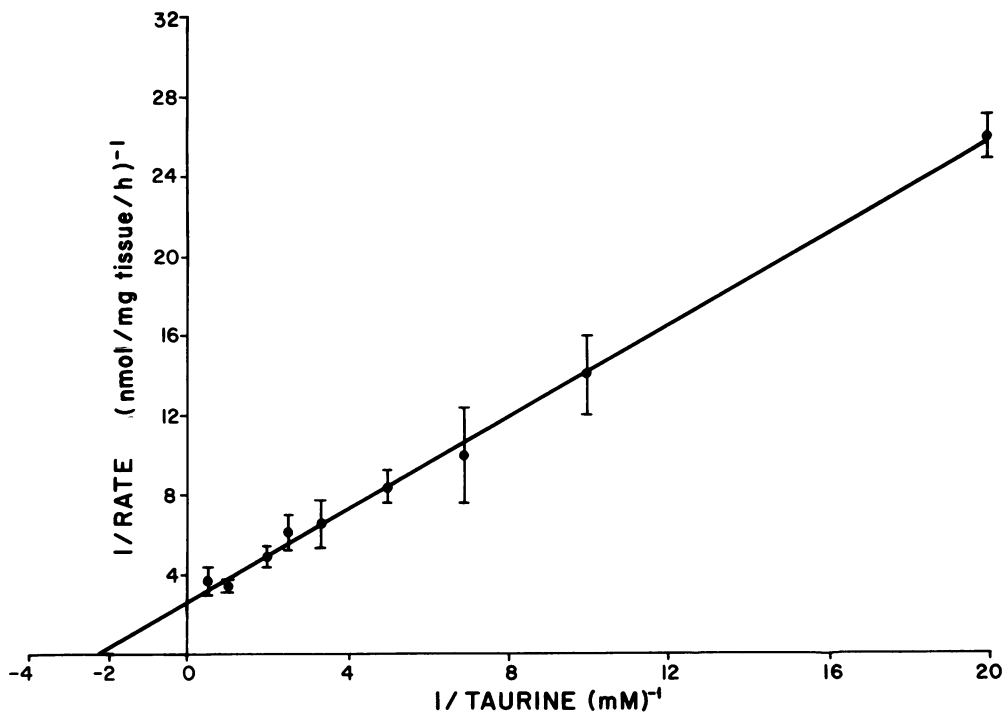
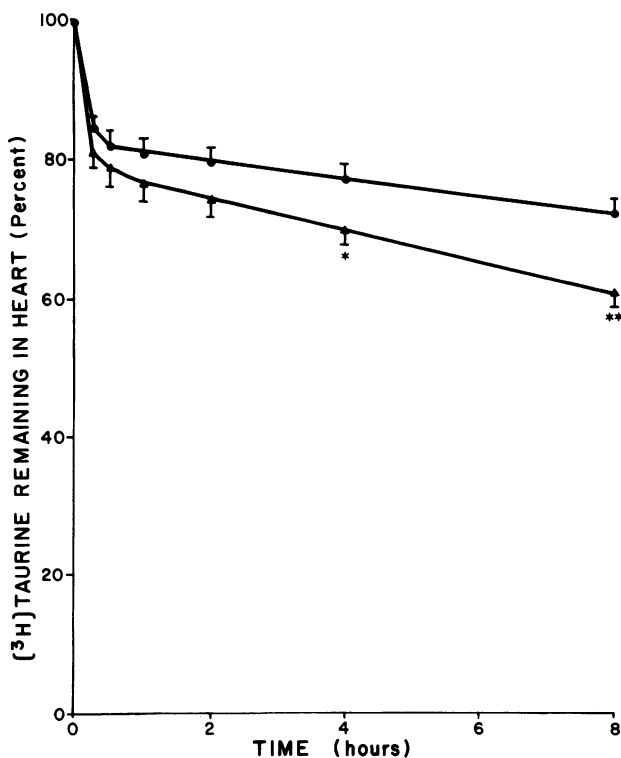


FIGURE 5 Determination of  $K_m$  and  $V_{max}$  for taurine uptake. The data represent the means  $\pm$ SEM for the net sodium-dependent uptake of taurine shown in Fig. 4. The line is the "best-fit" according to a least-squares linear regression analysis.



deprivation did not significantly affect uptake in either case. Depletion of endogenous energy supplies in the absence of glucose is known to require incubation for greater than 4 h (18). Therefore, it is not surprising that the removal of glucose from the medium did not affect taurine uptake during the course of this experiment. The metabolic inhibitor, 2,4-dinitrophenol, reduced uptake by 80% from that of the control,  $P < 0.001$ .

*Inhibition of taurine uptake by analogues.* Structurally related compounds that were tested for their ability to inhibit taurine uptake into the fetal mouse heart are shown in Table II. The hearts were incubated

FIGURE 6 Countertransport of taurine. Hearts were incubated individually for 2 h with 0.01 mM  $[^3\text{H}]$ taurine after which the medium was removed and the hearts rinsed and placed in new culture dishes with 0.35 ml of either taurine-free medium (●) or medium containing 1 mM taurine (▲). At intervals from 15 min to 8 h the medium was removed from each heart and  $[^3\text{H}]$ taurine measured. Fresh medium was added, and the incubation continued. The  $[^3\text{H}]$ taurine in the hearts at the end of 8 h was added to that which had appeared in the medium at the various time points and normalized. Each point represents the mean for four hearts, bars denote SEM. \*, Significantly different from control,  $P < 0.05$ , based on Student's  $t$  test. \*\*, Significantly different from control,  $P < 0.01$ , based on Student's  $t$  test.

for 1 h with 0.2 mM [<sup>3</sup>H]taurine and 20 mM inhibitor.  $\beta$ -Amino acids such as hypotaurine (H<sub>2</sub>N-CH<sub>2</sub>CH<sub>2</sub>-SO<sub>2</sub>H), which differs from taurine only in the oxidation state of the sulfur moiety, and  $\beta$ -alanine were the most effective compounds in decreasing taurine uptake, reducing it by 87% ( $P < 0.001$ , for both compounds).  $\gamma$ -Aminobutyric acid inhibited uptake by 32%,  $P < 0.01$ .

The  $\alpha$ -amino acids, alanine,  $\alpha$ -aminoisobutyric acid, glycine, leucine, and threonine, did not inhibit taurine uptake when present at a concentration of 20 mM. Identical results were obtained when the experiments were conducted in either medium 199, which contains amino acids, or in amino acid-free MEM. The data from the two sets of experiments were combined in Table II. These amino acids were chosen because they are representative of groups of amino acids that are transported by three separate and distinct amino acid transport systems in the Ehrlich's ascites tumor cell (25, 26) and chick embryo heart cell (27).

A number of other compounds structurally similar to taurine were also tested for their ability to inhibit the accumulation of [<sup>3</sup>H]taurine. Guanidino-taurine and guanidinopropionic acid, the guanidiny-lated derivatives of taurine and  $\beta$ -alanine, respectively, were effective inhibitors reducing uptake by 75–80%. They were nearly as effective as hypotaurine and  $\beta$ -alanine. *N*-Acetyltaurine and isethionic acid did not inhibit uptake. Taken together, these observations suggest that the amino group of taurine is required for transport. In addition, there appear to be restrictions on the size or charge characteristics

TABLE I  
Energy Dependence of [<sup>3</sup>H]Taurine Uptake

Treatment	Uptake*
	pmol/mg tissue/h
Glucose/O <sub>2</sub> deprivation‡	
5 mM Glucose + O <sub>2</sub>	338 ± 17
O + O <sub>2</sub>	388 ± 18
5 mM Glucose + N <sub>2</sub>	96 ± 8§
O + N <sub>2</sub>	73 ± 15§
Metabolic inhibitor <sup>¶</sup>	
Control	549 ± 25
0.25 mM DNP	110 ± 5¶

\* Mean ± SEM,  $n = 3$  per group.

‡ Incubations were carried out in MEM with or without 5 mM glucose under 95% O<sub>2</sub>/5% CO<sub>2</sub> or 95% N<sub>2</sub>/5% CO<sub>2</sub>, [<sup>3</sup>H]taurine = 0.2 mM.

§ Significantly different from glucose + O<sub>2</sub>,  $P < 0.001$ .

¶ Incubations were carried out in medium 199 (control) or medium 199 with 2,4-dinitrophenol (DNP) under 95% O<sub>2</sub>/5% CO<sub>2</sub>, [<sup>3</sup>H]taurine = 0.2 mM.

¶ Significantly different from control,  $P < 0.001$ .

TABLE II  
Inhibition of Taurine Uptake by Taurine Analogues and Amino Acids

Inhibitor*	Uptake as percent of control‡
Hypotaurine	13.1 ± 1.1§ (4)
$\beta$ -Alanine	13.4 ± 1.6§ (4)
$\gamma$ -Aminobutyric acid	67.9 ± 4.4 <sup>¶</sup> (4)
$\alpha$ -Alanine	94.9 ± 0.9 (7)
$\alpha$ -Aminoisobutyric acid	105.7 ± 4.1 (5)
Glycine	109.6 ± 10.8 (3)
Leucine	103.3 ± 2.6 (5)
Threonine	104.8 ± 9.9 (6)
Guanidinopropionic acid	20.7 ± 0.7§ (5)
Guanidino-taurine	26.2 ± 2.8§ (3)
<i>N</i> -Acetyltaurine	110.6 ± 4.5 (4)
Isethionic acid	121.4 ± 5.1 (6)
2-Aminoethane phosphonic acid	83.2 ± 8.5 (4)
Aminomethane sulfonic acid	80.3 ± 5.5 (3)
3-Aminopropane sulfonic acid	89.2 ± 1.9 (4)

\* Incubations were carried out for 1 h with 0.2 mM [<sup>3</sup>H]taurine and 20 mM inhibitor. Controls were incubated with 0.2 mM [<sup>3</sup>H]taurine only.

‡ Controls were normalized to 100%. Values represent mean ± SEM. Numbers in parentheses indicate the number of hearts.

§ Significantly different from control,  $P < 0.001$ .

¶ Significantly different from control,  $P < 0.01$ .

of the acidic group because 2-aminoethane phosphonic acid was a very poor inhibitor, whereas  $\beta$ -alanine was a good inhibitor. The length of the carbon chain of the amino acid is also an important factor because the one carbon homologue, aminomethane sulfonic acid, and the 3-carbon analogues, 3-aminopropane sulfonic acid and  $\gamma$ -aminobutyric acid, were all relatively weak inhibitors of taurine uptake.

*Ouabain.* The effect of ouabain on the uptake of taurine was determined in three experiments summarized in Table III. Taurine uptake was increased in the presence of 1  $\mu$ M and 0.1 mM ouabain. The increases were significant both when comparisons were made on the basis of absolute values for uptake and on the basis of relative uptake. Ouabain at 10 nM did not significantly affect taurine uptake in either case.

## DISCUSSION

Our studies have demonstrated a carrier-mediated transport system for taurine in the fetal mouse heart, an in vitro model for the study of myocardial metabolism. The uptake was temperature dependent, saturable,  $K_m = 0.44$  mM, and exhibited specific structural requirements. It was characterized as a  $\beta$ -amino acid transport system that was sodium de-

TABLE III  
Effect of Ouabain on Taurine Uptake

Treatment*	Uptake	
	pmol/mg tissue/h†	% control‡
Control	124±8 <sup>a</sup>	100
10 nM	131±15 <sup>a,b</sup>	103±6
1 μM	143±14 <sup>b</sup>	114±4 <sup>¶</sup>
0.1 mM	162±18 <sup>c</sup>	129±7 <sup>¶</sup>

\* Hearts were incubated for 1 h in medium (control) or medium plus ouabain at the concentration shown. At the end of 1 h [<sup>3</sup>H]taurine was added to a final concentration of 0.2 mM to all hearts and the incubation was continued for an additional hour. The hearts were then removed and analyzed for [<sup>3</sup>H]taurine uptake. A total of 28 hearts, 7 hearts per group, from three separate litters were used.

† Mean±SEM. Groups designated with different letters were significantly different,  $P < 0.05$ , as determined by analysis of variance and Duncan's New Multiple-Range test.

‡ Mean±SEM.

§ Significantly different from control,  $P < 0.02$ , based on Student's *t* test.

¶ Significantly different from control,  $P < 0.01$ , based on Student's *t* test.

pendent, energy dependent, and able to accumulate taurine against a concentration gradient.

Taurine uptake systems have been studied in a variety of mammalian tissues including the kidney (14, 28), retina (29, 30), blood platelets (31, 32), brain (33, 35), and Ehrlich's cell (36). Preliminary reports also indicate that such a system is present in the heart (14–16). Our results extend previous observations of cardiac taurine uptake to demonstrate that a carrier-mediated transport system exists in the myocardium. They also demonstrate that taurine transport in the fetal mouse heart shares many properties with taurine transport in adult mammalian tissues.

The  $K_m$  of 0.44 mM obtained for taurine uptake in the fetal mouse heart corresponds closely to the values observed in the kidney (14, 28), Ehrlich's cell (36), retina (29), and the low-affinity systems in platelets (32) and the brain (34, 35). Methods for calculating the  $K_m$  deserve further comment because the total uptake of taurine by the fetal mouse heart cannot be accounted for solely on the basis of a single transport process. Taurine transport is not completely saturable at normal sodium concentrations (Fig. 4), nor does correction for diffusion, as estimated by uptake at 4°C (15), result in saturation kinetics. These data imply that a second uptake system exists. Similar phenomena have been reported for  $\beta$ -alanine (26) and taurine (36) uptake into the Ehrlich's cell. In these two cases, the nonsaturable component could be attributed to uptake by one or more of the processes that transport neutral  $\alpha$ -amino acids. However, the

affinity was very low, approximating a diffusion-controlled process, and could be corrected for mathematically (26, 36). This explanation for the nonsaturation of taurine uptake in the fetal mouse heart and the mathematical correction was previously reported (15). By taking advantage of the sodium dependence of the uptake system, we have obtained saturability and identified the nonsaturable component as sodium-independent uptake. The values of the kinetic constants for net sodium-dependent taurine transport were equivalent to those obtained previously (15) by the mathematical correction of total uptake for the nonsaturable process.

Accelerative exchange diffusion or countertransport (25) is a characteristic of many biological transport systems. This phenomenon was observed for taurine (Fig. 6), demonstrating that the carrier will transport taurine in both directions across the cell membrane. This provides additional evidence for the existence of a carrier-mediated transport system for taurine in the fetal mouse heart.

In agreement with previous reports from studies of taurine uptake in retina (29, 30), platelets (31), kidney (14), heart slices (14), and brain (35), sodium was found to be required for maximal rates of transport. The demonstration of an energy requirement for uptake (uptake was reduced 70–80% either by oxygen deprivation or addition of 2,4-dinitrophenol) is a characteristic of taurine transport in the fetal mouse heart shared with noncardiac tissues (29–31, 35, 36).

A net increase in taurine content of 20% was observed in hearts incubated for 4 h in medium containing 0.5 mM taurine. Assuming a single intracellular pool, the intracellular concentration of taurine was calculated to be 25 mM. Thus, the net accumulation of taurine in the hearts was accomplished against a concentration gradient of approximately 50-fold. The validity of this assumption depends upon the currently available evidence which has failed to show that taurine is compartmentalized within the cell (13, 24, 37). In brain, which exhibits uptake characteristics for taurine similar to heart (13), over 90% of the taurine is present in the cytoplasm, and that portion present in subcellular organelles appeared to be freely exchangeable with taurine in the cytoplasm (24). The ability of the system to concentrate taurine against an apparent concentration gradient in conjunction with the demonstrated energy dependence of the system suggests that the sodium-dependent transport of taurine into the fetal mouse heart is an active process.

We have demonstrated a mechanism for taurine transport in the fetal mouse heart that is separate and distinct from known neutral  $\alpha$ -amino acid transport processes (25, 27) based on the differential affect of  $\alpha$ -amino acids and  $\beta$ -amino acids on taurine uptake. Investigations of taurine uptake in the



Ehrlich's cell (25, 36) and  $\beta$ -alanine uptake in the Ehrlich's cell (26), kidney (38), and intestine (38) have utilized the same criterion to describe these processes as specific for  $\beta$ -amino acids and separate from the  $\alpha$ -amino acid transport systems.

Analogues of taurine that were the most effective inhibitors of uptake were the  $\beta$ -amino acids that retained charged groups at both ends of a three-atom skeleton. In contrast,  $\alpha$ -amino acids representative of the known neutral  $\alpha$ -amino acid transport systems of the Ehrlich's cell (24) and chick embryo heart cell (26) were totally ineffective as inhibitors of taurine uptake. In addition, variations in chain length, removal or blockage of the amino group, and the substitution of a phosphonic acid moiety for the sulfonic acid group markedly altered the ability of a compound to inhibit taurine uptake.

The significance of the increase in taurine uptake in the presence of ouabain under the conditions of these experiments is difficult to assess at the present time. Ouabain may act directly on the taurine transport system either to increase uptake or decrease efflux. Alternatively, the effects may be secondary to the actions of ouabain on ion movements in the myocardium (39). Previous studies have reported an interaction between taurine and cardiac glycosides based on the ability of taurine to mitigate digitalis toxicity (4, 5). Further investigation of the relationship between taurine and cardioactive drugs will be required before the stimulation of taurine uptake by ouabain can be placed in proper perspective.

In this investigation we have presented the first detailed characterization of taurine uptake in the mammalian myocardium. Inasmuch as the synthesis of taurine by the heart is not a significant contributor to cardiac taurine stores, uptake may represent the major controlling factor in the elevated levels of taurine observed in pathophysiological conditions of the heart (3-8). Care must of course be exercised in extrapolating biochemical or pharmacological responses observed in studies of fetal tissue *in vitro* to the adult animal. However, the similarities outlined above between taurine transport in this model of the mammalian myocardium and several tissues from the adult animal suggest that the fetal mouse heart can serve as a model system for the investigation of factors which regulate taurine uptake into the heart. The availability of a model system of the mammalian heart with a demonstrable system for taurine influx and efflux will make possible detailed studies of the affect of taurine on cardiac metabolism and interactions between taurine and cardioactive drugs.

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