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TAURINE TRANSPORT BY RAT HEPATOCYTES IN PRIMARY CULTURE

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Summary

Hepatic taurine concentration is 30—100 times that of plasma, suggesting an efficient taurine uptake mechanism in the hepatocyte. The characteristics of hepatic taurine transport were studied in primary cultures of adult rat hepatocytes. Taurine uptake was concentrative and linear for over 4 h. At taurine concentrations 2.5—100 μM, uptake was saturable with constants $K_m = 44$ μM, $V = 0.28$ nmol/mg protein per min and $E_A = 13.2$ kcal/mol. Uptake was inhibited 41% by incubation under N_2 and was competitively inhibited by β-alanine ($K_i = 94$ μM) and hypotaurine ($K_i = 14$ μM). Uptake was linearly dependent upon Na^+ concentration from 0 to 140 mM. A second non-saturable uptake process was identifiable only at taurine concentrations greater than 1 mM. This process was presumed to represent passive diffusion. At taurine concentrations existing in plasma, taurine enters the hepatocyte primarily by a single, Na^+-dependent, carrier-mediated, oxygen-requiring transport process.

Introduction

The liver is of major importance in mammalian taurine metabolism. It is the first organ presented with absorbed dietary taurine; in many mammals the liver synthesizes taurine; and in mammals which conjugate bile acids with taurine, the liver serves as a major excretory route for taurine. In rats, hepatic taurine equilibrates rapidly with plasma taurine [1,2]. The hepatic taurine pool has rapid turnover compared to that of muscle or central nervous system taurine

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

pools [1,2]. Liver taurine in the rat [3] and dog [4] can be depleted by infusing unconjugated bile acid which is rapidly conjugated with taurine and excreted. In man, biliary obstruction increases hepatic taurine [5] and diseases which increase requirements for bile acid conjugation decrease liver taurine as manifest by a high glycine to taurine-conjugate ratio in the bile acid pool of such patients. Metabolic conditions in the liver most likely determine how much and how rapidly dietary taurine is distributed to the less-labile peripheral storage pools.

In keeping with its importance in taurine metabolism, the liver probably has mechanisms for rapid uptake and accumulation of taurine. Plasma taurine concentrations in man are reported to range between 25 and 150 μM [6], whereas liver taurine concentrations are about 2.0 μmol/g wet (or about 2900 μM intracellular fluid) [5]. Perfused rat and cat livers concentrate taurine to 15—100 times plasma concentrations (unpublished observations). The present report describes taurine transport characteristics of isolated rat hepatocytes in primary non-replicating culture.

Materials and Methods

Taurine (grade A) and hypotaurine were purchased from Calbiochem, La Jolla, CA, U.S.A. Hepes buffer, sodium lauryl sulfate, choline chloride, choline bicarbonate, β-alanine and collagenase (C2139) were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. [1,2-^{14}C]taurine was purchased from New England Nuclear, Boston, MA, U.S.A. Eagle's basal medium with Hank's salts was purchased from Grand Island Biological Co., Grand Island, NY, U.S.A.

Methods

Rat hepatocytes were isolated by the collagenase perfusion technique [7] as follows. Non-fasted male Sprague-Dawley rats, 150—250 g, were anesthesized with ether and the liver exposed with a midline abdominal incision. The inferior vena cava just above the right renal artery was ligated and the portal vein cannulated. Infusion of calcium-free Hank's balanced salt solution was begun, the thorax was rapidly opened and the thoracic inferior vena cava cannulated. 100 ml of this solution was infused over about 15 min and effluents discarded. Next, 0.5% collagenase in 50 ml Hank's balanced salt solution containing 3 mM $CaCl_2$ was perfused and recirculated for 20 min. All solutions were infused at 37 °C. The liver was then removed, minced into 25 ml of the collagenase solution, and shaken gently for 10 min. Supernatant fluid containing parenchymal and non-parenchymal hepatic cells was removed and placed into 10 ml Eagle's basal medium containing 16% heat-inactivated horse serum to which had been added 20 mM HCO_3^-, 10 mM Hepes buffer, 250 U insulin, 100 000 U penicillin G; 100 mg streptomycin and 250 μg amphotericin. Collagenase solution was added to the liver residue and again shaken for 10 min. The supernatant was treated as before. The two supernatant tubes were placed in a refrigerated centrifuge at 4 °C, the centrifuge started and switched off when speed reached 500 $\times g$. Supernatant containing mostly non-parenchymal cells was aspirated and the pellet washed twice with 20 ml of Eagle's

basal medium. Cells were combined, rewashed, and resuspended in about 20 ml Eagle's basal medium. 100 μl of the suspension was placed in 1 ml of a solution of 0.9% NaCl with 2.5 mg/ml trypan blue. Cells were counted in a standard hemacytometer chamber. Cells excluding trypan blue and cells permeable to trypan blue were counted separately. Yields ranged from 80 to 150 · 10^6 cells per liver with greater than 90% trypan blue exclusion. Cell suspensions were made to contain 350 000 cells/ml; 1.5 ml was plated per 35 mm culture dish and dishes were incubated in 95% air/5% CO_2 at 37 °C. Cumulative uptake of taurine was linear for at least 4 h of incubation for cells incubated in Hank's balanced salt solution containing 100 μM [1,2-^{14}C]taurine. All studies were performed on cells which had been in culture for 72 h.

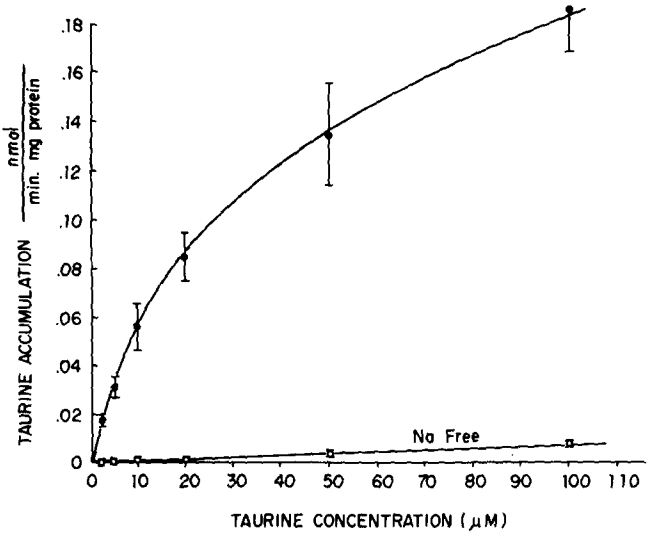
Transport studies. Plates were removed from the incubator and washed twice with phosphate-buffered saline at 37 °C. Excess fluid was blotted and 1.0 ml of incubation fluid, heated to 37 °C, was added. Except where otherwise stated, incubation fluid was Hank's balanced salt solution containing 20 mequiv./l Hepes buffer. Appropriate substrate and inhibitors were added to the fluid before addition to the plates. Plates were gently agitated (40 cycles/min) in a Dubnoff shaker at 37 °C until incubation was complete. Plates were removed, washed rapidly three times with 0.9% NaCl at 4 °C, excess fluid blotted, and cells lysed with 1 ml of a 1% aqueous solution of sodium lauryl sulfate. Lysed cells were dispersed by aspiration through a 25 Ga needle. Two aliquots, 100 μl each, were taken for determination of protein by the micro-method of Lowry et al. [8] and one aliquot of 500 μl was placed into Bray's solution for measurement of radioactivity. Counting was performed in a Beckman LS-230 scintillation counter. Quench was corrected by the channels-ratio method.

Since uptake of taurine by hepatocytes was linear for over 4 h, a convenient time interval of 30 min was selected for all incubations. Values were expressed as nmol taurine accumulated/mg protein per min. All plates were run in triplicate. In preliminary experiments, taurine in cell lysate was separated from taurine-conjugated bile acids by butanol extraction. Over 98% of counts appeared in the taurine fraction. This fraction was carried through our standard taurine assay procedure [3]. Over 90% of the counts appeared in the taurine band of the cellulose-polyacetate electrophoretic strip. These data indicate that taurine was not being significantly metabolized by the hepatocytes during the course of incubation.

All counts in the cell homogenate were considered to be intracellular. Kletzien et al. had demonstrated in primary liver cell cultures that three washes leaves negligible amounts of extracellular fluid [9]. Intracellular fluid volume in our hepatocytes was determined by the method of Kletzien et al. [9]. It was found to be 1.91 μl/mg protein, a value similar to that determined by other investigators.

Results

Fig. 1a shows taurine uptake velocity plotted against taurine concentration, 2.5—100 μM, in the incubation fluid. The curve has the properties of a saturable process and when the data are plotted in a double-reciprocal fashion



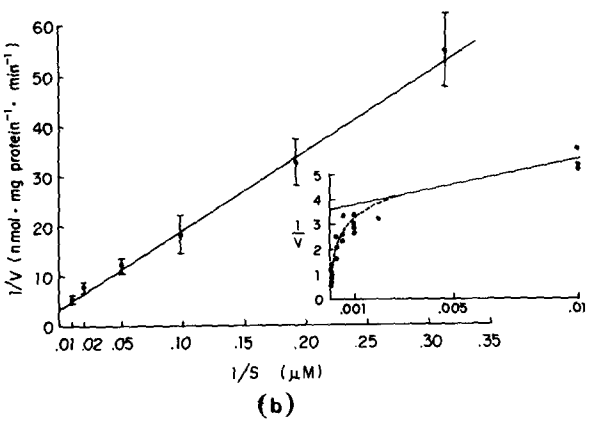


Fig. 1. (a) Taurine accumulation with varying substrate concentrations. Cells were incubated at 37°C in Hank's balanced salt solution for 30 min. In the lower curve, choline chloride and choline bicarbonate were substituted for sodium salts. Vertical bars represent ± S.D. of five independent experiments. (b) Double-reciprocal plot of data from (a). The insert demonstrates that at high substrate concentrations, 10^3—$2 \cdot 10^4$ μM, a non-saturable process becomes evident.

they describe a straight line (Fig. 1b). The constants of the process are K_m = 44.2 μM and V = 0.28 nmol/mg protein per min. The insert to Fig. 1b shows that a second, non-saturable, process also contributes to taurine uptake at high incubation fluid taurine concentrations (10^3—$2 \cdot 10^4$ μM).

The saturable uptake process is sodium dependent. Fig. 1a also shows taurine uptake velocity when hepatocytes were incubated in a medium in which choline bicarbonate and choline chloride were substituted for the respective sodium salts. Taurine transport was inhibited by over 95% in the absence of Na^+. Thus a sodium-independent process recently described by

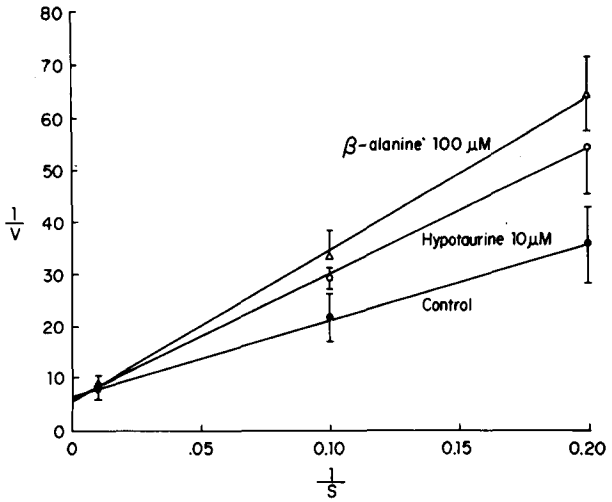


Fig. 2. Competitive inhibition of taurine uptake by β-alanine and hypotaurine. Incubations were at 37°C for 30 min in Hank's balanced salt solution. Inhibition concentrations were: β-alanine, 100 μM, and hypotaurine, 10 μM. Vertical bars represent mean ± S.D. of five independent experiments.

Grosso et al. in fetal mouse heart [10] appears to contribute little to taurine uptake in primary cultures of adult rat hepatocytes. Taurine transport was linearly dependent upon sodium concentration from 0 to 140 mM, as described by several authors in other tissues [10,11].

The uptake process was competitively inhibited by compounds known to inhibit taurine transport in other tissues. Fig. 2 shows the effect of 100 μM β-alanine and of 10 μM hypotaurine upon uptake of 100 μM taurine. The inhibition constant (K_i) for β-alanine was 94.0 μM; that for hypotaurine was 14.2 μM. Substances found not to inhibit uptake of taurine included 1000-μM solutions of glycine, taurocholate, glycocholate, ornithine, and isethionic acid.

Temperature dependence of taurine transport was determined by incubating cells in three concentrations of taurine (100, 1.0 and 2.5 μM) at three temperatures (37, 22 and 4°C). The Arrhenius plot was linear and yielded and activation energy of 13.2 kcal/mol. This is within the accepted range for carrier-mediated transport processes [12,13]. Oxygen dependence was demonstrated by incubating cells under N_2 in Hank's balanced salt solution previously saturated with N_2. Taurine accumulation was inhibited by 41%.

The metabolic toxins, sodium azide, 2,4-dinitrophenol, and sodium cyanide were tested for their ability to inhibit taurine transport. Preincubation of cells in media containing these toxins in concentrations up to 0.1 mM caused no inhibition of transport. At higher concentrations, such as those previously reported to be toxic to transport processes, sufficient cytotoxicity occurred so that a large portion of protein was lost from the plate. Although some inhibition was evident at these concentrations, we are unable to interpret these data.

Carrier-mediated transport processes usually manifest the phenomenon of counter transport. We preincubated cells for 3 h in Hank's balanced salt solu-

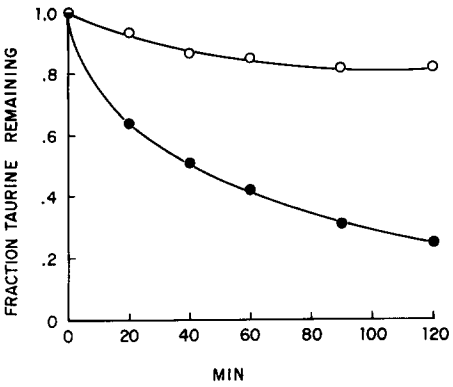


Fig. 3. Countertransport of taurine. All cells were preincubated in Hank's balanced salt solution containing 10 μM [1,2-^{14}C]taurine for 3 h. Media were changed to Hank's balanced salt solution containing either no taurine or 1000 μM taurine and plates incubated at 37°C. At 20-min intervals plates were washed and cells assayed for residual taurine. ●———●, 1 mM taurine; ○———○, control.

tion containing 10 μM [1,2-^{14}C]taurine. Cells were then washed and placed in media containing either no taurine or 1 mM unlabelled taurine. Groups of three plates were removed and washed at 20-min intervals for 120 min, and the percent [1,2-^{14}C]taurine remaining in the cells determined. Fig. 3 shows the results. At 120 min, cells incubated in 1 mM taurine retained only 25% of their [^{14}C]taurine whereas control cells retained 82%.

Discussion

Although characteristics of taurine transport have been well described for cardiac muscle [10,11], various tissues of the central nervous system [14—17], blood platelets [18,19] and kidney [11,20], they have not been described for the liver. Because of the anatomic complexity of the organ, accurate studies of amino acid transport in normal adult hepatocytes were impossible until techniques were developed for isolating parenchymal cells in suspension or in primary culture. Both of these preparations have now been used successfully to study transport processes [21,22]. In the present report we define the characteristics of taurine transport in primary non-replicating cultures of adult rat hepatocytes.

Our data indicate that taurine uptake in the adult rat hepatocyte is a single, carrier-mediated, sodium-dependent, active transport process. The uptake process is similar to that described by Kromphardt [23] in Ehrlich ascites tumor cells, except that this author did not investigate the sodium dependence of the process. The structural specificity of the transport site seems similar to that in other tissues. The pattern of competitive inhibition is compatible with β-amino acid transport site requiring a molecule with both an electronegative and an electropositive end separated by the equivalent of two methylene groups with the two-carbon chain length optimal [18]. We sought competition by glycine and taurocholate not because of structural homology, but because of physiological considerations. Our data suggest that availability

of either glycine or taurine for bile acid conjugation is not determined by mutual competition for transport across the hepatocyte membrane. Similarly, high levels of taurine-conjugated bile acids probably cannot inhibit bile acid taurine conjugation by blocking uptake of taurine into the hepatocyte.

Most data concerning taurine transport in various tissues indicate that the saturable uptake process is sodium dependent. Thus, sodium-dependent saturable systems for taurine uptake have been demonstrated in rat brain synaptosomes [16,17,24], frog retina [25], human blood platelets [18,19], mouse kidney slices [20], rat heart and kidney slices [11], as well as in the present work with adult rat hepatocytes. An exception to this appears in a recent report of taurine transport in rat brain synaptosomes [17]. The authors found two saturable transport systems for taurine with different affinities. The low-affinity system appeared only partially sodium dependent.

In addition to saturable, sodium-dependent taurine uptake, most tissues exhibit non-saturable, sodium-independent uptake which becomes significant usually only at high taurine concentrations. Whether this represents diffusion only or whether it also represents sharing of a neutral α-amino acid transport system is uncertain. Both Kromphardt [23] and Christensen [26], working with Ehrlich ascites tumor cells, concluded that β-alanine uptake, but not taurine uptake, was in part attributable to the neutral α-amino acid transport system. Christensen suggested that a large part of the non-saturable taurine uptake occurred by simple diffusion [27]. Grosso et al., on the other hand, found a rather large component of non-saturable, sodium-independent taurine uptake in the fetal mouse heart: about 20% of taurine uptake at 200 μM substrate concentration was sodium independent [10]. Because the magnitude of this process was greater than could be accounted for by diffusion, the authors suggested, but did not demonstrate, that it was attributable to taurine transport by the neutral α-amino acid transport system. In the present work, the very small non-saturable component of taurine uptake most closely resembles that in Ehrlich ascites tumor cells.

Taurine uptake in the adult hepatocyte has at least one other similarity to uptake in the Ehrlich ascites tumor cell. Active uptake is by a single carrier-mediated transport process. Such is not the case for renal and neural tissue and human platelets. In normal and hypertaurinemic mouse kidney, Chesney et al. described two sodium-dependent saturable uptake systems for taurine, one with a K_m value in the 0.2 mM range and a second with a K_m value in the 17 mM range [20]. Most studies in neural tissue indicate more than one saturable uptake process for taurine. Two transport systems have been reported in rat brain synaptosomes [17,24], and brain cell membranes [14], although the presence of more than one saturable process has been disputed [15]. Three saturable systems for taurine transport with K_m values from 2.9 to 360 μM have been described by Gant and Nauss in human blood platelets [18]. Nonetheless, several other tissues studied demonstrate only one saturable uptake system for taurine. Such tissues include Ehrlich ascites tumor cells [23,26], fetal mouse heart [10], and adult rat liver. It is possible, however, as suggested by Hruska et al. [24], that very high-affinity systems may be overlooked unless very low substrate concentrations of taurine (approx. 0.5 μM) are used. Thus far, such high-affinity systems (K_m values less than 5 μM) have been identified

only in neural tissue and platelets. Considering plasma taurine concentrations in mammals, it is unlikely that such a high-affinity system would be physiologically important in parenchymal, non-neural tissue.

In summary, taurine uptake in adult rate hepatocytes appears less complex than uptake in neural and renal tissue. It is mediated via a single, saturable, sodium-dependent, active process most similar to that described in Ehrlich asites tumor cells.

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