PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 18–22 (1980)

The Influence of Hypotaurine on Taurine Transport in Isolated Renal Cortex Tubules (40817)[1]

ROBBEN GINGERY AND RUSSELL W. CHESNEY

Department of Pediatrics, The University of Wisconsin Center for Health Sciences, Madison, Wisconsin 53792

Taurine (2-aminoethanesulfonic acid) is synthesized from cysteine via oxidation to cysteine sulfinic acid which undergoes a further decarboxylation to yield hypotaurine (2-aminoethanesulfinic acid) (1). As well, the oxidation of cysteamine from pantothenic acid yields hypotaurine. Hypotaurine, a metabolic intermediate is then oxidized to form taurine, although no enzyme to facilitate this oxidation has been detected in mammalian tissue (2). Taurine is found in large quantities in various organs of mammals and probably serves as a neurotransmitter (3).

Taurine is lost from the body primarily in the urine and its renal tubular reabsorption occurs via the β-amino acid transport system. Heterogeniety of renal taurine accumulation is found in both mouse (4) and rat cortex slices (5) and in isolated cortex tubules (6). This uptake of taurine is sodium-dependent and occurs on both a low K_m and high K_m system. A single uptake system is present in isolated tubular luminal membranes (7). The interaction of hypotaurine, the sulfinic acid analog of taurine, with taurine accumulation has been examined in brain (8) and platelets (9), but its influence on renal taurine transport has not been characterized. In this study we examine the effect of hypotaurine on taurine uptake at both K_m sites in isolated rat renal cortex tubules so as to better define the nature of the renal β-amino acid transport system.

Materials and methods. Rat renal cortex tubules were isolated from adult (180–240 g) and 4-week-old Sprague–Dawley rats by a previously described method (6, 10). Collagenase II (Worthington Biochemical Corp.) was used to separate tubules and all glassware used was freshly siliconized. Taurine uptake was determined by a previously described method (4–6) and tissue was incubated in Krebs–Ringer bicarbonate medium gassed continuously with 95% O_2–5% CO_2. At various time intervals, aliquots of constantly suspended tubules were removed, separated from medium by centrifugation at 16,000 rpm in a Sorvall RC-5B centrifuge and the amount of taurine accumulated was measured. The accumulation of [1,2-^{14}C]taurine was determined from the ratio of counts per minute per milliliter intracellular fluid (ICF) to counts per minute per milliliter extracellular fluid (ECF) by a standard formula to provide this isotopic distribution ratio (11). Medium and boiled tissue supernatant was measured in Aquasol (New England Nuclear) as described (4–6). The percentage ICF (55%) and ECF (24.8%) were determined as previously described using radiolabeled polyethylene glycol (6, 12).

Taurine efflux was measured in tubules incubated for 30 min to load tissue and then placed in taurine-free medium. Samples were removed at various times and the radiolabeled taurine content was ascertained.

Quantitative measurement of chemical taurine was made by a previously described technique (13) utilizing the reaction of the primary amine group of taurine with fluorescamine (Hoffmann–LaRoche) to form a fluorescent compound that could be measured in a fluorometer (GK Turner and Sons). Taurine was first separated from hypotaurine by passing protein-free supernates of tissue through a Dowex 50W-X12 ion-exchange resin column as previously described (13). Radiolabeled [1,2-^{14}C]-taurine (sp act 56.4 μCi/μmole) was obtained from New England Nuclear. Unlabelled hypotaurine was obtained from

[1] Supported in part by U.S. Public Health Service Grants AM-19489 and Research Career Development Award AM-00421 (RWC).

0037-9727/80/050018-04$01.00/0

Vega Biochemicals. Tubule experiments were performed in triplicate and data comparison was made with Student's t test and regression analysis.

Results. Taurine accumulation at 10 μM and 2 mM both during initial uptake (1–10 min) and at steady state (20–30 min as shown previously (6)) was inhibited when hypotaurine was present (Table I). After 30 min incubation, the taurine distribution ratio at 10 μM, transported predominately by the low K_m, high affinity system (6), was 39.4 ± 1.4 (SE) (n = 36). After a similar time, taurine accumulation at 2.0 mM, transported predominately by the high K_m, low affinity system, was 5.85 ± 0.26 (SE) (n = 12). Taurine accumulation at 10 μM was blocked by a level of hypotaurine as low as 5 μM and was progressively inhibited as the hypotaurine concentration in the incubation medium was increased (Table I). A similar situation was found for uptake at 2 mM. The high K_m system is more effectively inhibited by hypotaurine as shown in Table I.

The nature of this inhibition is shown in Fig. 1. It is apparent that hypotaurine inhibits the uptake of taurine over the range of 10 to 200 μM in a competitive fashion. The K_m for taurine uptake over this range was 125 μM and the V_{max} was 4.15 μmole/ICF/30 min, similar to values previously reported by us in slices (5, 13) and isolated tubules (6). The value for K_i derived from the three inhibitor concentrations used (100 and 500 μM and 5.0 mM hypotaurine) ranged from 70 to 170 μM indicating that hypotaurine had a greater affinity for the taurine uptake site than we found previously for β-alanine (4, 5).

We have previously examined taurine accumulation in immature animals and found β-alanine inhibition of uptake by cortex slices at both sites of a comparable degree to that found in adult rats (13). Hypotaurine also blocked taurine uptake at 10 μM by cortex from immature (4-week-old) rats. The control distribution ratio after 30 min incubation in tubules from these animals was 61.9 ± 2.1. Inhibition of accumulation by hypotaurine was 44.7 ± 1.8% at 50 μM, 57.2 ± 0.5% at 100 μM, and 79.8 ± 1.3% at 500 μM, quite similar to the values found in adult tubules. The influence of hypotaurine on uptake at 2 mM was not examined.

Taurine efflux was examined after a 30-min incubation with 10 μM taurine. Tubules were washed and then placed in a taurine-free medium or a medium containing 100 μM hypotaurine. Taurine efflux was significantly enhanced in the presence of hypotaurine at times after 2.5 min ($P < 0.01$ to <0.001). The percentage remaining taurine was always lower in the presence of

TABLE I. HYPOTAURINE EFFECT ON TAURINE ACCUMULATION

10 μM Taurine[a]			2 mM Taurine[b]		
Hypotaurine concn μM	Percentage inhibition[c]	Statistical significance	Hypotaurine concn mM	Percentage inhibition	Statistical significance
0	0	—	0	0	—
5	11.1 ± 2.0	< 0.05	0.5	18.3 ± 1.8	< 0.001
10	16.4 ± 2.0	< 0.01	2.0	33.7 ± 2.5	< 0.001
50	40.4 ± 2.2	< 0.001	5.0	57.3 ± 2.5	< 0.001
70	60.0 ± 0.7	< 0.001	10.0	68.9 ± 1.6	< 0.001
100	63.4 ± 1.1	< 0.001			
500	72.2 ± 1.3	< 0.001			
5000	94.1 ± 2.0	< 0.001			

[a] Cortex tissue was incubated in medium with hypotaurine level shown and 10 μM taurine for 30 min. At least six determinations of uptake for each hypotaurine level were made and the mean taurine accumulation in control (0 hypotaurine) had a distribution ratio of 39.4 ± 1.4.

[b] Tissue was incubated for 30 min with 2.0 mM taurine and six determinations were made. The mean taurine accumulation in control (0 hypotaurine) had a distribution ratio of 5.85 ± 0.26 (SE).

[c] Mean ± SE.

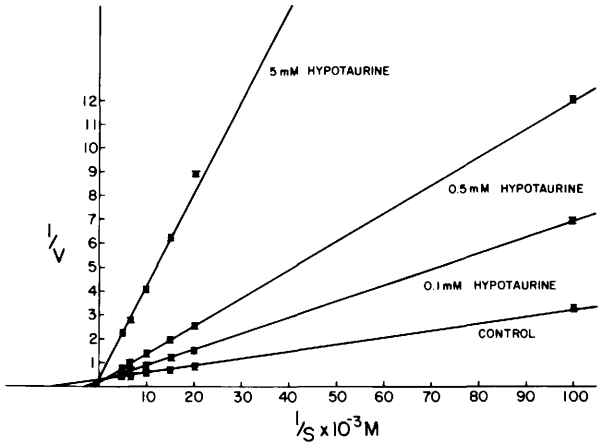

FIG. 1. A Lineweaver–Burk plot of taurine accumulation by isolated cortex tubules over the taurine concentration range of 10 to 200 μM. The hypotaurine concentrations used were as shown. V represents velocity of transport (micromoles per milliliter intracellular fluid per 30 min). Each point shown is the mean ± SE of at least six determinations.

hypotaurine (Fig. 2). The rate of taurine exit into the hypotaurine-free medium was 0.0064 μmole/min/g tissue and into the hypotaurine-containing medium was 0.015 μmole/min/g. These results indicate counterflux of taurine by hypotaurine. The influence of hypotaurine on efflux from the high K_m system was comparable and counterflux was found (data not shown).

It is possible that hypotaurine was being converted to taurine under the conditions of the incubation and that the inhibition of taurine accumulation found was related to this metabolic conversion. Accordingly, the levels of chemical taurine in isolated tubules were determined by direct fluorescamine assay after 1, 30, and 60 min incubation in either 10 μM taurine and 100 μM hypotaurine or 10 μM taurine alone (Table II). After 1 min there was no difference in tissue taurine content, but at 30 and 60 min the taurine levels were lower in the presence of hypotaurine. Since tissue taurine content was lower when the inhibitor was present, hypotaurine was probably not converted to taurine. Had this conversion

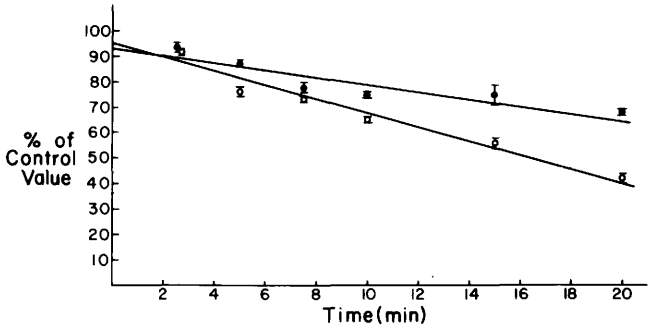

FIG. 2. The efflux of taurine from isolated cortical tubules previously incubated in a medium containing 10 μM taurine. Tubules were then washed and placed in a taurine-free medium. Each point shown is the mean ± SE of at least six determinations. At the time shown tissue was removed and the taurine content was measured as described in the text. The closed circles indicate efflux into a taurine-free medium; open circles indicate efflux into a taurine-free, hypotaurine-containing medium. The rate of taurine exit is given in the text.

TABLE II. TISSUE TAURINE CONTENT AFTER INCUBATION[a]

Time (min)	Taurine level (μmole/g tissue)[b]		
	Taurine 10 μM	Taurine 10 μM + hypotaurine 100 μM	P
1	2.19 ± 0.14	2.30 ± 0.05	NS
30	3.15 ± 0.41	1.76 ± 0.09	< 0.05
60	1.69 ± 0.02	1.06 ± 0.19	< 0.02

[a] Incubation in Krebs–Ringer medium containing 10 μM taurine for the times shown at 37°C. Tissue taurine content was ascertained by a fluorescamine assay after Dowex 50 column chromatography (13). The levels of taurine shown represent the amount found in the tubule pellet after centrifugation at 16,000 rpm at 4°C for 10 min.
[b] Mean ± SE of six experiments.

taken place, a higher or comparable tissue taurine concentration would be expected. Moreover, the lower tissue taurine content in the presence of hypotaurine was consistent with the findings of enhanced efflux after incubation with this inhibitor.

Tissue taurine was higher in control tubules at 30 min than at 1 min at a $P < 0.05$ using a paired t test. Accordingly, accumulation of taurine occurred in the absence of hypotaurine.

Discussion. It has previously been shown that other β-amino acids inhibit the transport of taurine by renal epithelium in man (14), mouse (4), and rat (5–7). Both β-alanine and β-aminoisobutyric acid will prevent the accumulation of taurine in slices (4, 5, 13), isolated tubules (6), isolated renal brush border vesicles (7), and, as well, *in vivo* (4) and under conditions of continuous microperfusions (15). Taurine can also inhibit β-alanine uptake in renal slices (16) and in brush border membrane vesicles (17). Hypotaurine will also inhibit taurine transport. Rozen *et al.* (7) found that 4 m*M* hypotaurine blocked taurine uptake at 37 μ*M* by brush border vesicles by 99.3%. Our findings in isolated tubule segments indicate that hypotaurine blocks taurine accumulation at concentrations transported predominately by the low K_m (10 μ*M*) and high K_m (2.0 m*M*) systems, as previously defined (6). Equimolar hypotaurine is actually a more effective inhibitor when taurine is predominately transported in the high K_m system (Table I). It is likely that taurine accumulation and interaction with hypotaurine is being examined at the basolateral membrane when isolated tubules are used for these transport studies. The K_m values for taurine uptake by tubules at both sites is similar to the values reported by us in slice studies, a technique that examines basolateral organic solute uptake (6, 13). Moreover, the K_m for taurine accumulation by the single uptake system in brush border membranes is 17 μ*M* (7), quite dissimilar from the K_m values we find in tubules.

The K_i for hypotaurine on taurine transport over the range 10 to 200 μ*M* is 70 to 170 μ*M*, lower than the K_i for β-alanine inhibition of 0.8 m*M* we previously found (5). Moreover using 5.0 m*M* hypotaurine, we find 94% inhibition of uptake by the low K_m system, comparable to findings in brush border membranes (7). Uptake in immature animal cortex is also inhibited.

Hypotaurine inhibition of low K_m site taurine uptake is competitive as was previously found both in platelets (9) and brain synaptosomes (18). As Gaut (9) suggests the structure of both β-alanine, the carboxylic acid analog of taurine, and hypotaurine, the sulfinic acid analog, provides the ideal spatial configuration and molecular length to block the taurine transport site.

Further evidence of taurine-hypotaurine interaction in renal epithelium is the enhanced efflux of taurine found after incubation in hypotaurine. Of interest is that the efflux of taurine shown in Fig. 2 is linear with time, rather than a log-linear efflux into trans-zero medium. Efflux of taurine from slices is log linear as we have previously shown (4, 5, 13), but efflux from tubules appears to be linear (6). Linear

efflux of taurine shown in Fig. 2 is linear since this method is free from the diffusional constraints found when using the slice method.

It seems unlikely that conversion of hypotaurine to taurine by cortex tissue accounts for this inhibition since tissue taurine levels are lower after incubation with hypotaurine following 30 and 60 min incubation. In addition, no enzymatic oxidative process for the conversion of hypotaurine to taurine has been found (2). The lower tissue level of taurine after 60 min probably represents efflux after prolonged incubation, a finding we have previously noted (6). In conclusion it appears that hypotaurine is a useful taurine analog to examine the kinetics of the β-amino acid transport system in several tissues including the renal epithelium and is an inhibitor of taurine transport with a high affinity for both transport sites.

Summary. The effect of hypotaurine on taurine transport in isolated tubules from rat renal cortex was examined at the low K_m (10 μM) and the high K_m (2 mM) system. Taurine transport is inhibited at both sites, but hypotaurine appears to inhibit accumulation on the high K_m system to a greater extent. The effect is competitive and hypotaurine has greater affinity for the taurine uptake system than does β-alanine. Finally, hypotaurine is shown to interact with the taurine carrier so that exchange diffusion is observed.

1. Awapara, J., *in* "Taurine" (R. Huxtable and A. Barbeau, eds.), p. 1. Raven, New York (1976).
2. Cavallini, D., Scandurra, R., Dupre, S., Federici, G., Santero, L., Ricci, G., and Barra, D., *in* "Taurine" (R. Huxtable and A. Barbeau, eds.), p. 59 Raven, New York (1976).
3. Jacobsen, J. G., and Smith, L. H., Jr., Physiol, Rev. **48**, 424 (1968).
4. Chesney, R. W., Mohyuddin, F., and Scriver, C. R., J. Clin. Invest. **57**, 183 (1976).
5. Chesney, R. W., and Jax, D. K., Biochim. Biophys. Acta **489**, 83 (1977).
6. Friedman, A. L., Jax, D. K., and Chesney, R. W., Renal Physiol., in press.
7. Rozen, R., Tennenhouse, H. S., and Scriver, C. R., Biochem. J. **180**, 245 (1979).
8. Lahdesmaki, P., and Oja, S. S., J. Neurochem. **20**, 1411 (1973).
9. Gaut, Z. N., and Nauss, C. B., *in* "Taurine" (R. Huxtable and A. Barbeau, eds.), p. 91 Raven, New York (1976).
10. Burg, M. B., and Orloff, J., Amer. J. Physiol. **203**, 327 (1962).
11. Mohyuddin, F., and Scriver, C. R., Amer. J. Physiol. **219**, 1 (1970).
12. Chesney, R. W., Jax, D. K., Mohyuddin, F., and Scriver, C. R., Renal Physiol. **1**, 166 (1979).
13. Chesney, R. W., and Jax, D. K., Pediatr. Res. **13**, 854 (1979).
14. Scriver, C. R., Pueschel, S., and Davies, E., N. Engl. J. Med. **274**, 636 (1966).
15. Dantzler, W. H., and Silbernagel, S., Pfluegers Arch. **367**, 123 (1976).
16. Nutzenadel, W., and Scriver, C. R., Amer. J. Physiol. **230**, 643 (1976).
17. Hammerman, M., and Sacktor, B., Biochim. Biophys. Acta **509**, 338 (1978).
18. Hruska, R. E., Huxtable, R. J., and Yamamura, H. I., *in* "Taurine and Neurological Disorders" (A. Barbeau and R. J. Huxtable, eds.), p. 109, Raven, New York (1978).

Received October 5, 1979. P.S.E.B.M. 1980, Vol. 164.