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HETEROGENEITY OF THE β-AMINO-PREFERRING TRANSPORT SYSTEM IN RAT KIDNEY CORTEX

DIFFERENTIAL INFLUENCE OF GLUTATHIONE OXIDATION

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Summary

Taurine, a naturally found β-amino acid, is inert in rat renal cortex slices. Its active accumulation by slices is abolished by anaerobiosis, a strongly acidic media or the removal of Na^+. Concentration-dependent uptake studies reveal more than one taurine carrier: the apparent K_m value for uptake below 1.1 mM is 0.4 mM and the apparent K_m value above 1.1 mM is 14.5 mM. Of all amino acids tested only β-alanine, another β-compound, inhibited uptake. The oxidizing agent diamide was used to lower the concentration of GSH in rat cortex slices. The ability to accumulate taurine by the low K_m system was decreased in diamide-treated slices, but not by the high K_m system. Diamide was found to greatly augment efflux of taurine taken up from lower concentrations but not from higher concentrations. GSH in the media prevented this diamide-induced inhibition of uptake and enhanced efflux at lower taurine concentrations. A possible mechanism of diamide inhibition of uptake is that intracellular GSH depletion leads to greatly enhanced efflux of taurine, thus preventing uptake.

Introduction

Taurine (2-aminoethanesulfonic acid) is an amino acid characteristic of rodent urine and is a major pathway for sulfur excretion in the rat [1,2]. Certain inbred mouse strains excrete even greater quantities of this β-amino acid [3]. The intraperitoneal injection of other β-amino acids will augment taurine excretion [4], thus suggesting an in vivo group-specific renal tubular transport system. Other studies have clearly established a tubular transport system in vivo

Abbreviation: EGTA, ethylene glycol-bis-(β-aminoethylether)-*N*,*N*'-tetraacetic acid.

with specific affinity for β-amino compounds [5—7]. We have recently investigated the renal epithelial transport of taurine in three inbred mouse strains and have examined in detail the kinetics of in vitro accumulation [8]. The renal transport characteristics of this β-amino compound have not been explored in great detail [9] in the rat, nor has taurine interaction with other α-amino acids been examined, although the uptake of another β-amino compound, β-alanine, has been recently examined in rat kidney [10].

Taurine was chosen as the representative agent for the β-amino group since it is an abundant component of the amino acid pool of most tissue [11] and, in mouse kidney, is an inert substance with no renal metabolism [8]. Isotopic tracer studies of taruine, like those using α-aminoisobutyric acid or α-methyl-D-glucoside, specifically define uptake characteristics. It is also known that taurine is largely free within renal tubular cells and not incorporated into protein, thus its uptake measures transport specifically [2,11].

The studies in mouse kidney slices revealed heterogeneity of taurine uptake with a low K_m (high affinity) and high K_m (low affinity) component [8]. These studies were interpreted to show at least two saturable uptake systems for transport across the basilar membrane of the renal tubule, as the kidney slice technique used results in occlusion of luminal membranes and selective exposure of basilar membranes to substrate in the media [12]. Nutzenadel and Scriver [10] have also suggested that there may be heterogeneity of β-amino compound uptake in the rat, but the extent of correlation of uptake pathways in both rodent classes is not known. In the present study, we have found evidence for heterogeneity of uptake by cortex slices in rat kidney.

In a series of experiments aimed at defining the role of glutathione (GSH) oxidation on taurine transport, we have also found that diamide, an oxidant of intracellular GSH in the red cell [13], behaved differentially with respect to its inhibition of taurine uptake. Diamide, the trivial name for dizene dicarboxylic acid bis-(N_1,N_1-diamethylamide), reacts with GSH in a stoichiometric fashion. Its action is rapidly reversed by the addition of GSH or other thiol reagents [14]. Although diamide is primarily a GSH oxidant, it can interact with protein-bound SH groups and reduced pyridine nucleotides [15]. The uptake of several α-amino acids and α-methyl-D-glucoside by rat renal cortex slices is inhibited by diamide; however, this inhibition can be overcome by the addition of GSH [16,17]. These investigations have not examined the influence of diamide on more than one uptake system. Our studies have shown that diamide appears to inhibit the low K_m system far more than the high K_m system, implying that GSH is more protective at this site in kidney cortex.

Methods

Animals. Adult male Sprague-Dawley rats weighing between 220 and 300 g were obtained from Holtzman Co., Madison, Wisc. from the ARS Sprague-Dawley Co., Madison, Wisc. Animals were caged separately and fed on standard Lab-Blox Chow. Animals were killed by decapitation and kidneys were rapidly removed and placed in chilled media.

In vitro investigations. Thin cortex slices (0.2 mm) were prepared from rat kidney and incubated by a previously described technique [18,19], except that

a hand manufactured microtome was utilized that was devised to provide thinner slices than is possible with a conventional Stadie Riggs microtome. The average slice width is 0.2 mm. Two slices weighing between 4 and 6 mg each were put into a Warburg flask or open mouthed 15-ml vials in order to measure uptake or oxidation of substrate. The slices were incubated in 2.0 ml Tris-Ringer glucose or Krebs-Ringer phosphate glucose buffer (300 mosM, pH 7.4, at 37°C under 100% O_2 at 7 l/min). After incubation, slices were withdrawn, blotted, weighed on a torsion balance and placed in 1.0 ml distilled water boiled for 5 min. The supernatant was recovered and placed in a scintillation vial containing Aquasol (New England Nuclear). Released CO_2 was trapped in filter paper soaked with 50% KOH and was counted in a water and Aquasol system [18].

The percentage total tissue water was ascertained by drying tissue to constant weight at 110°C for 48 h. The percentage extracellular water was determined by the poly[^{14}C]ethylene glycol method [16]. The percentage intracellular water was derived by subtracting the percentage extracellular fluid from the percentage total tissue water. The uptake of substrate was determined by a previously described method [18].

Inhibition studies were accomplished by incubation of tissue in the presence of substitute and inhibitor. Other amino acids were present at 10 times the substrate concentration. Equimolar choline chloride or KCl were substituted for NaCl in studies of sodium dependence.

Efflux studies were performed by the method of Segal et al. [20] wherein tissue was incubated in substrate-containing media until a steady state was achieved. Tissue was then removed, blotted, weighed and placed in substrate-free efflux media. The rate of appearance of taurine in the medium was measured.

Three experimental designs were used to examine the influence of diamide on taurine uptake. First, slices were incubated in media at 37°C with substrate and diamide. Secondly, slices were preincubated with media alone or with media containing diamide for 15 min in flasks on ice at 4°C and then removed, blotted and transferred to a flask containing 2 ml media and substrate for incubation at 37°C. In these experiments both Tris-Ringer glucose and Krebs-Ringer phosphate buffer was used. Thirdly, slices were preincubated in diamide and media or media alone at 4°C for 15 min, washed in media for 30 min at 25°C, blotted and incubated at 37°C [16,17]. All media was adjusted to pH 7.4 unless otherwise noted.

Analysis. All slice experiments were performed in triplicate and data was compared using the Student's *t*-test.

Corrected values for uptake of taurine on more than one system were obtained by the methods of Scriver and Mohyuddin [21] and Neal [22]. Analysis of uptake kinetics were performed under steady-state conditions [21] unless otherwise indicated.

Materials. [1-^{14}C]Taurine (spec. act. 4.5 Ci/mol) and poly[1,2-$^{14}C_2$]ethylene glycol (spec. act. 2.3 Ci/mol) were purchased from New England Nuclear, Boston, Mass. Radiochemical purity was confirmed by appropriate one-dimensional chromatography.

Unlabeled amino acids, diamide and glutathione were purchased from Sigma

Chemical Co., St. Louis, Mo. All chemicals used to prepare media were reagent grade.

Results

Tissue water content. The extracellular space is 25.3 ± 1.5% (mean ± S.D., n = 22); the comparable total tissue water is 80.2 ± 3.5% and the intracellular space, by derivation, is 54.9% of the weight of the slice.

Time-course of uptake. Taurine uptake by renal cortex slices reaches steady state after 45 min at 1.1 and 20.0 mM external media taurine concentration, but continues up to 120 min at 0.01 and 0.11 mM. This indicates that influx and efflux are equal at a later time at taurine levels <1 mM.

Less than 1% of radiolabeled [^{14}C]taurine is transformed to CO_2 during a 120 min incubation of cortex slices. Therefore, taurine appears to be inert in rat kidney cortex and the isotropic distribution ratio can be envisioned as being equivalent to the chemical distribution ratio.

Further, taurine is taken up against a chemical distribution gradient indicating active accumulation.

Concentration-dependent uptake. Uptake of taurine is affected by the extracellular concentration (Fig. 1); the uptake distribution ratio falls as the substrate concentration is raised. Uptake does not follow simple Michaelis kinetics throughout the concentration range 0.001—60 mM (Fig. 2). Since steady state is reached after 45 min at 1.1 and 20 mM taurine, but not until 120 min at 0.01 and 0.11 mM, concentration-dependent studies were performed at 60 min for all concentrations and after 120 min at taurine levels <1.0 mM. The shape of the Eadie-Augustinsson plot and the values for K_m were similar whether the tissue was incubated for 60 or 120 min. The method of Neal [22] was used to obtain a $K_{m,1}$ = 0.4 mM and a V = 3.8 μmol uptake/ml tissue intracellular fluid per h and a $K_{m,2}$ = 15.2 mM and a V = 45 μmol/g per h. The derived v vs. v/s

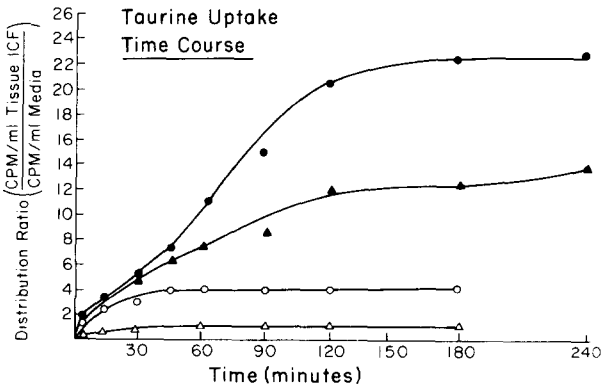

Fig. 1. Time course for uptake of [1-^{14}C]taurine by rat kidney outer cortex slices at 37°C in pH 7.4 Tris buffer. The influence of taurine concentration on uptake ratio is also shown. The conversion of taurine after uptake to CO_2 or other labeled material is <1% of the total uptake. Thus, isotopic and chemical distribution ratios are comparable. Data are the mean of at least 12 determinations at each point. ●, 0.012 mM/l; ▲, 0.1 mM/l; ○, 1.012 mM/l; △, 20 mM/l. ICF, intracellular fluid.

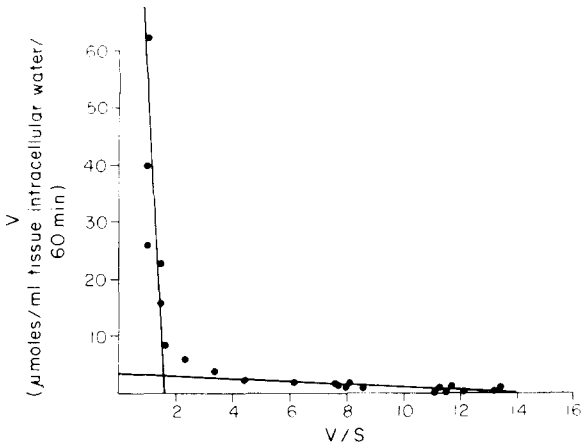

Fig. 2. Concentration-dependent kinetics for taurine uptake by rat kidney cortex slices examined over a 60 000-fold variation in substrate concentration (0.001—60 mM). The Eadie-Augustinsson transformation reveals more than one mode of uptake. Data are the composite of several hundred determinations over the total range of taurine concentration.

(Eadie-Augustinsson) transformation of uptake data led to a biphasic regression plot.

Efflux. Slices were preloaded with taurine and efflux into taurine-free media was examined over 16 min at 37°C. The slope of the efflux curve differed when slices loaded at 0.11 mM were compared to slices loaded at 1.11 mM. The efflux from tissue with an intracellular fluid concentration of 0.3 μmol/ml is 0.007 μmol/ml intracellular fluid per min and with an intracellular fluid level of 2.3 μmol/ml is, 0.054 μmol/ml intracellular fluid per min.

Inhibitors of taurine uptake. Boiling of tissue, incubation under nitrogen, exposure to acid conditions (pH 5.0), 10^{-2} M NaCN and 10^{-3} M iodoacetate completely blocked active taurine accumulation by cortex slices. Removal of sodium from the external media with the substitution of choline or potassium abolished concentrative accumulation. At 0.01 mM taurine, the isotopic distribution ratio was 12.1 ± 0.6 S.D. (n = 12) in the presence of sodium, and 1.1 ± 0.1 S.D. (n = 12) whenever choline or potassium was used to replace sodium ($P < 0.001$). As choline or potassium was serially substituted for sodium, there was increasing inhibition of taurine uptake. This inhibition was more apparent on the low K_m uptake system, probably since isotopic distribution ratios are higher.

The interaction between taurine and several α-amino acids β-alanine was studied. 10-fold higher levels of glycine, leucine, cystine, glutamic acid and tyrosine altered taurine uptake by <0.01% (n.s.). Only β-alanine inhibited the uptake of taurine. The inhibition by β-alanine was competitive on both the low K_m and high K_m uptake systems. A K_i = 0.8 mM β-alanine was calculated for the low K_m uptake system (Fig. 3). No K_i was calculated for the high K_m system.

Since β-alanine and taurine appear to have a common transport system the influence of extracellular β-alanine on intracellular taurine was examined by counterflow. Taurine efflux is enhanced by external β-alanine as further evidence of a shared system.

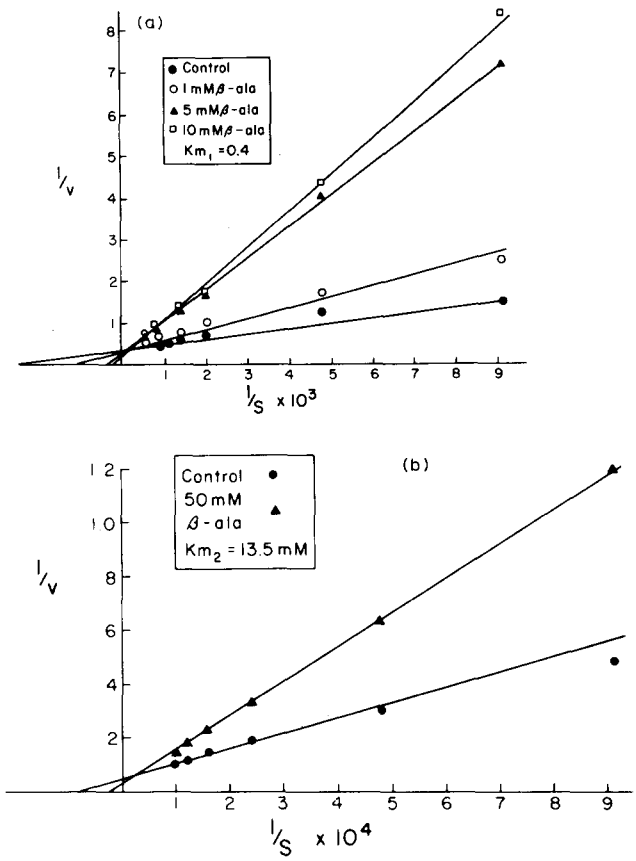

Fig. 3. (a + b) A conventional Lineweaver-Burk transformation of uptake data on the low K_m (A) and high K_m (B) system by rat kidney cortex slices reveals competitive inhibition. Shown in the insert are the values for $K_{m,1}$ and $K_{m,2}$. Data points are the mean of six determinations.

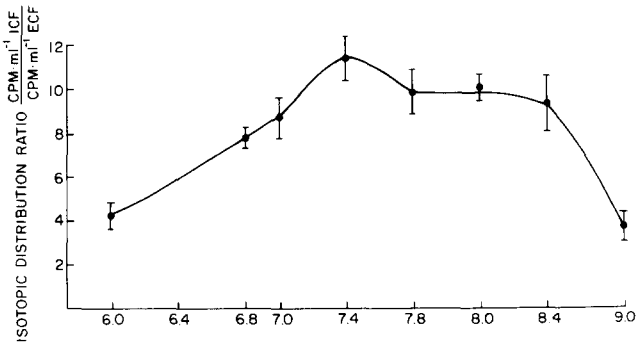

Fig. 4. The uptake of [1-^{14}C]taurine for 60 min accommodated on the low K_m system (0.01 mM) at various pH values in the media. Similar observations were obtained at the high K_m system (2.11 mM). Data represents the mean and S.D. of six determinations. ICF, intracellular fluid; ECF, extracellular fluid.

pH dependence. The uptake of taurine is maximal at pH 7.4 on both the low K_m and high K_m system (Fig. 4).

Diamide. Diamide inhibits taurine uptake in dose-related fashion that is maximal at a diamide concentration of 9 mM. 9 mM diamide inhibits taurine uptake by 85% at 0.01 mM (low K_m system) and by 46% at 1.11 mM (high K_m system). 9 mM diamide did not inhibit uptake at 20 mM taurine. The influence of diamide on the low K_m uptake system is greater than on the high K_m system as seen from an Eadie-Augustinssen plot of the uptake data (Fig. 5). Preincubation of tissue with diamide at 4°C will still prevent taurine uptake even though the tissue is placed in a diamide-free media for uptake studies (Table I).

The addition of equimolar GSH to diamide in the preincubation step will largely prevent the diamide-related inhibition. This prevention by GSH is dose related, and requires at least equimolar GSH to achieve maximal prevention. It is also apparent from the time course of taurine uptake with diamide and GSH that diamide inhibits the low K_m system more fully (Fig. 6).

Is is possible that diamide may prevent taurine uptake by altering the energy metabolism of the cell and negating the normal sodium gradient between intracellular and extracellular fluid. Taurine uptake at 0.01 mM was inhibited by 40% after preincubation in diamide (30 mM), but was inhibited by 90% if the second incubation media contained 20 mM iodoacetate. Similarly taurine uptake is inhibited after diamide preincubation by 40%, but no concentrative uptake is found if the second incubation media has choline substituted for sodium. Both 20 mM iodoacetate and sodium-free media prevent concentrative

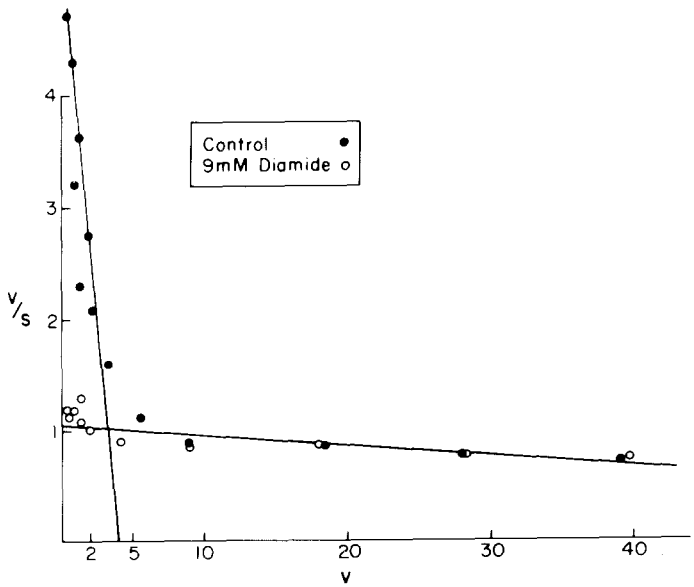

Fig. 5. Concentration-dependent kinetics for taurine uptake by rat kidney cortex slices examined over a 400-fold variation in substrate concentration. The Eadie-Augustinsson transformation reveals only one mode of uptake in the presence of 9 mM diamide. Data are the composite of 120 determinations over the total range of taurine concentrations.

TABLE I

TAURINE ISOTOPIC DISTRIBUTION RATIO

Renal cortex slices were preincubated at 4°C in medium alone (control) or in 0.024 M diamide and then incubated with taurine at 37°C and pH 7.4 under 100% O_2 for 60 min. Diamide was washed free in medium at 25°C (b, c). Each value is the mean ± S.D. of the data (n = Nos. of experiments).

Taurine concentration	Condition	Control	0.024 M diamide	P
0.01 mM	(a) Krebs-Ringer phosphate medium	5.62 ± 0.82 (n = 14)	2.41 ± 0.64 (n = 14)	0.001
	(b) Tris-Ringer phosphate medium diamide washed free at 25° C	8.02 ± 0.68 (n = 12)	3.61 ± 0.46 (n = 12)	0.001
	(c) Krebs-Ringer phosphate medium diamide washed free at 25° C	7.94 ± 1.66 (n = 15)	2.57 ± 0.33 (n = 25)	0.001
1.11 mM	(d) Krebs-Ringer phosphate medium	2.24 ± 0.42 (n = 27)	1.33 ± 0.26 (n = 24)	0.01
20.1 mM	(e) Krebs-Ringer phosphate medium	1.10 ± 0.08 (n = 4)	1.08 ± 0.09 (n = 4)	n.s.

n.s., not significant.

taurine uptake when diamide is not present. These results suggest that diamide does not block taurine uptake on the low K_m system as does the metabolic poison iodoacetate or a sodium-free state. Finally the tissue sodium concentration of slices incubated in the presence and absence of diamide, left for 12 h in HNO_3 and measured by flame photometry is the same.

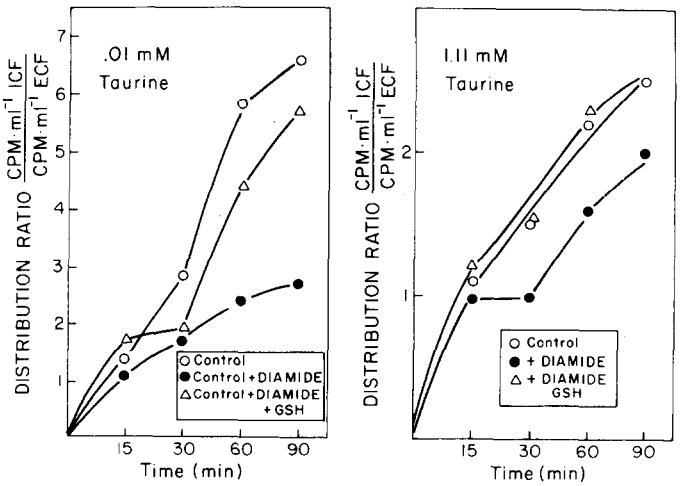

Fig. 6. Uptake of [1-^{14}C]taurine at various times by rat kidney cortex slices accomodated predominantly on the low K_m (0.01 mM) and the high K_m system (1.11 mM). Uptake is significantly decreased by diamide only at 0.01 mM taurine concentration in the media. Uptake in the presence of diamide and equimolar GSH is not different from uptake by control slices at 1.11 mM. Data are the mean of at least 12 determinations. ICF, intracellular fluid; ECF, extracellular fluid.

TABLE II

TAURINE UPTAKE DISTRIBUTION RATIO IN CALCIUM-FREE MEDIA

Tissue slices were preincubated for 15 min at 4°C in taurine-free media with or without 20 mM diamide. Media is Ca^{2+}-free Krebs-Ringer phosphate, except in I, and contains high magnesium (II, III) or EGTA (IV). Slices were then removed, blotted, and placed in fresh media with 0.01 mM taurine and incubated at 37°C for 60 min. In III, slices were washed free of diamide in medium at 25°C for 15 min prior to incubation in taurine. The pH of all flasks was 7.4. Each value is the mean ± S.D. and n = Nos. of flasks. In II, the tissue was incubated with diamide throughout the experiment. The values in control in I are significantly different from II by $P < 0.001$, from III by $P < 0.05$ and from IV by $P < 0.01$.

Condition	I		II		III		IV	
	Krebs-Ringer phosphate	P	—Ca^{2+} + 2.5 mM Mg^{2+}	P	—Ca^{2+} + 2.4 mM Mg^{2+}	P	—Ca^{2+} + 2 mM EGTA	P
Control	4.91 ± 0.92 (n = 13)	<0.001	3.22 ± 0.51 (n = 5)	<0.001	3.81 ± 0.69 (n = 6)	<0.005	3.92 ± 0.36 (n = 12)	<0.001
Tissue + 20 mM diamide	2.41 ± 0.28 (n = 7)		1.17 ± 0.14 (n = 10)		2.49 ± 0.42 (n = 12)		2.76 ± 0.40 (n = 24)	

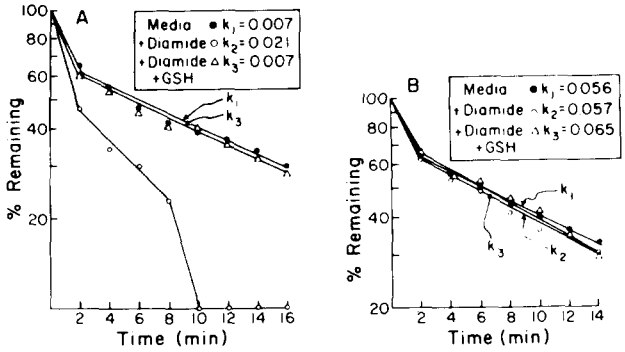

Fig. 7. Efflux of [1-^{14}C]taurine into pH 7.4 Tris-Ringer medium free of taurine at 37°C at low (A) and high (B) concentrations from rat kidney cortex slices preloaded with taurine so that the concentration is similar in the presence of medium alone or with diamide. Rates are calculated as μmol/min per g wet weight. At lower taurine concentration, pool depletion is increased in the presence of diamide, but efflux is similar from higher taurine concentrations. Data are the mean of at least six determinations at each point.

Efflux studies demonstrate that diamide greatly increases taurine efflux from tissue taken up by the low K_m site; this increased efflux can essentially be prevented by the addition of equimolar GSH (Fig. 7a). There is no influence of diamide on taurine efflux at the high K_m site; efflux into media alone, media with diamide and media with diamide plus equimolar GSH displays the same slope (Fig. 7b).

Various thiol reagents, including diamide, have been shown to alter calcium uptake by isolated mitochondria [23,24]. The influence of these agents can largely be reproduced by the addition of Mg^{2+} or EGTA to a calcium-free media. Therefore, taurine uptake was examined in a Ca^{2+}-free media in the presence of high Mg^{2+} or EGTA; 20 mM diamide was then added in these media to look for further inhibition of taurine uptake (Table II). In all instances diamide still further suppressed taurine uptake. Moreover, the extent of diamide suppression in each Ca^{2+}-free media is similar to the suppression found in normal Ca^{2+}-containing media. These studies suggest that diamide action on taurine uptake is not related to its inhibition of mitochondrial transport function. Further, the effect of calcium removal from the media is not as marked as with sodium removal. When GSH alone is incubated with taurine it will inhibit taurine uptake; this effect is also dose related.

When GSH alone is incubated with taurine it will slightly inhibit taurine uptake; this effect is also dose related. 2.0 mM GSH inhibits taurine uptake (0.01 mM) by 21% and 1.0 mM by 7%. However, if tissue is preincubated with GSH and then transferred to a GSH-free media this inhibitory effect is lost. This is in contrast to the findings with diamide preincubation, where transfer to a diamide-free media does not prevent inhibition of taurine uptake.

Discussion

It is known that the taurine concentration in rat kidney ranges from 7.1—11.0 μmol/g wet weight [11,25,26]. The concentration in plasma is 0.2 μmol/

ml [26,27]. Thus an in vivo distribution ratio of 35—55 is found in rat kidney. comparable to values reported in the mouse [8]. This study only examines the in vitro uptake and efflux of taurine from slices of rat renal cortex, since the in vivo characteristics have previously been described [7]. These slices studies specifically inform us about antiluminal membrane transport of this β-amino acid.

There is hetergeneity of taurine uptake by Sprague-Dawley rat cortex slices. The low K_m site has a K_m = 0.4 mM taurine and the high K_m site of 13--15 mM, values akin to those in the mouse [8]. A similar presentation in rat renal cortex sites for β-alanine uptake was found witha low K_m value of 0.15 mM and a high K_m value of 3.1 mM indicating a higher affinity for β-alanine uptake than for taurine uptake [10]. Unfortunately, the studies of Awapara and Berg [9] did not examine taurine uptake over a range of concentrations that would demonstrate the two uptake sites.

One difference between the two uptake systems is that taurine uptake by the low K_m system continues for 120 min and thus differs from that accumulated by the high K_m system. These differences may represent the time necessary to fill the intracellular taurine pool from a reduced extracellular taurine concentration. Steady state also may be reached more slowly at the low K_m site since the efflux constant is proportionately higher from tissue incubated in 0.11 mM taurine (0.007 μmol/g per min) than at a 10-fold higher concentration 1.11 mM where it is 0.054 μmol/g per min. Further, efflux of this inert β-amino acid at reduced intracellular taurine levels is greater than that found in the mouse.

It appears that β-alanine and taurine share both uptake systems. β-Alanine inhibits taurine uptake at each site in a competitive fashion. This finding is in contrast to the situation obtaining in the glycine-imino acid uptake pathways that are shared only at the high K_m site [18]. For reasons that are not readily apparent, β-alanine did not inhibit taurine uptake at a 2.5-fold higher concentration in the studies of Awapara and Berg [9]. Another study found taurine inhibition of β-alanine uptake at both carrier sites [10]. The rapid and complex metabolism of β-alanine demonstrated in mouse [8] and rat kidney cortex [10] may explain the failure of Awapara and Berg [9] to find an inhibition of kidney transport. This brisk utilization of β-alanine could also explain the rather high K_i found necessary to prevent taurine uptake in the rat, since our studies and those of Awapara and Berg [9] were not performed in the presence of (aminooxy) acetic acid an agent that effectively blocks β-alanine metabolism by preventing its transamination [8,10].

One test that has been used to distinguish various uptake sites is the variability of response to inhibitors [28]. We have found that diamide, effectively distinguishes between the two uptake sites. The studies using this thiol agent were originally undertaken to demonstrate that GSH depletion or reduction could inhibit amino acid uptake by means other than interference with the γ-glutamyl cycle [29]. This cyclic pathway has been proposed to catalyze the translocation and release steps of amino acid uptake [30]. However, taurine, as a β-amino acid cannot act as an acceptor of the γ-glutamyl moiety in the transpeptidase step and therefore any inhibition of taurine uptake by diamide treatment would imply that glutathione oxidation interferes with transport by a separate mechanism.

Taurine uptake on the low K_m system is inhibited by diamide and diamide

greatly increases taurine efflux at the low K_m site, but not the high K_m site. Therefore diamide serves as a means of separating each system. GSH oxidation may explain this selective inhibition since the studies of Hewitt et al. [16] demonstrated that incubation in 30 mM diamide for 5 min lowered GSH levels in Sprague-Dawley rat kidney cortex slices by 90%. This oxidized GSH could be intracellular [13] or membrane bound [15]. There are alternative explanations for this inhibition of taurine uptake as diamide also inhibits membrane-bound protein kinases [17] and reduced pyridine nucleotides [15] in intact nucleated mammalian cells. This potential oxidation of reduced pyridine nucleotides could result in altered mitochondrial calcium transport [24]. Studies in the absence of calcium with either added magnesium or EGTA demonstrate that diamide will inhibit taurine uptake at a time when mitochondrial calcium transport is decreased; thus this mechanism is unlikely.

It is possible that diamide may prevent low K_m system taurine uptake by affecting the energy metabolism of renal tubular cells thus changing the sodium gradient which may be the driving force for taurine transport. Diamide does not alter uptake or efflux of taurine at the high K_m site. However, this site is also sodium dependent, so if taurine altered the sodium gradient it should not affect one system preferentially. After preincubation in diamide there is still some active taurine accumulation at the low K_m site (50% of control values) but concentrative uptake is blocked when diamide-incubated tissue is then placed in iodoacetate, a metabolic blocker, or sodium-free, choline-containing medium. Were energy metabolism blocked enough by taurine to change the sodium gradient, one would not expect concentrative taurine uptake. Finally, tissue sodium levels are unchanged in diamide-incubated tissues. Thus this mechanism appears unlikely.

It is entirely possible that diamide inhibits taurine uptake by greatly increasing efflux at low taurine concentrations. With the rapid efflux of taurine in the presence of diamide, the normal tissue uptake distribution ratio cannot be established. The efflux constant of 0.021 μmol/min per ml intracellular water in the presence of diamide is similar to the uptake of 0.02 μmol/min per ml intracellular fluid at 0.112 mM taurine and could account for a failure to achieve concentrative uptake. Moreover, this would imply that intracellular GSH, interacting at the inner membrane, is depleted leading to this rapid efflux. Whether diamide inhibits the uptake of other L-amino acids [12] and α-methyl-D-glucose [13] by increasing efflux is unknown since its influence on efflux was not examined in those studies.

We have demonstrated evidence for heterogeneity of taurine uptake by rat renal cortex slices. The low K_m site is saturable and would serve to facilitate transport in the concentration range of taurine in rat serum, 100—200 μmol/l [27]. The high K_m site is similar to the non-saturable taurine transport system found in Erlich ascites cells and rat jejunum by Christensen et al. [31]. The uptake and efflux of this β-amino acid at the low K_m site are altered by diamide, a GSH-oxidizing agent.

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