Threonine metabolism in isolated rat hepatocytes

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House, James D., Beatrice N. Hall, and John T. Brosnan. Threonine metabolism in isolated rat hepatocytes. Am
J Physiol Endocrinol Metab 281: E1300–E1307, 2001.—The removal of the 1-carbon of threonine can occur via threonine
dehydrogenase or threonine aldolase, this carbon ending up in glycine to be liberated by the mitochondrial glycine cleavage
system and producing CO₂. Alternatively, in the threonine dehydratase pathway, the 1-carbon ends up in α-keto-
butyrate, which is oxidized in the mitochondria to CO₂. Rat hepatocytes, incubated in Krebs-Henseleit medium, were in-

cubated with 0.5 mM L-[1-14C]threonine, and 14CO₂ production resulting from the oxidation of L-[1-14C]threonine in isolated rat hepatocytes,

was measured. Added glycine (0.3 mM) marginally suppressed threonine oxidation. Cysteamin (0.5 mM), a potent

inhibitor of the glycine cleavage system, reduced threonine oxidation to 65% of controls. However, α-cyano-

acinnamate (0.5 mM), a competitive inhibitor of mitochondrial α-keto acid uptake, reduced threonine oxidation to 35% of controls.

These data provided strong evidence that ~65% of threonine oxidation occurs through the glycine-independent threonine
dehydratase pathway. Glucagon (10⁻⁷ M) increased threonine oxidation and stimulated threonine uptake by these cells. In summary, the majority of threonine oxidation occurs through the threonine dehydratase pathway in rat hepatocytes, and threonine oxidation is increased by glucagon, which also increases threonine’s transport.

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METHODS AND MATERIALS

Animals. Male Sprague-Dawley rats (weighing 250–350 g), obtained from our institution’s breeding colony, were used for all studies. Rats were housed in polycarbonate cages, with wood shavings as bedding, and were exposed to a 12:12-h light-dark cycle with the lights coming on at 0800. Animals were allowed ad libitum. The protocols involving the use of animals were approved by our local Animal Care Committee, in accordance with the guidelines established by the Canadian Council on Animal Care (8).

Diets. Unless otherwise indicated, all animals received commercial rodent chow (20% crude protein; Agway rmH 3000 rat chow). For studies involving high-protein-fed rats, rats consumed (±3 days) a synthetic diet formulated to American Institute of Nutrition (AIN)-93G (26) specifications for energy, fat, and micronutrients but containing 60% casein. Energy density was maintained to that in the original AIN-93G diet by substituting the casein for the cornstarch, sucrose, and dextrinized cornstarch (removed in proportion to their concentration in the original formulation).

Isolation of rat hepatocytes. Rats were anesthetized by the intraperitoneal administration of pentobarbital sodium (Somnitol, 60 mg/kg; MTC Pharmaceuticals, Cambridge, ON, Canada). The isolation of hepatocytes from rat livers was performed as described previously (3). Cell viability was assessed by measuring the cellular exclusion of a 0.2% trypan blue solution and at all times was 95%. Hepatocytes were resuspended in Krebs-Henseleit medium equilibrated with O2-CO2 (95:5) and containing 2.5% bovine serum albumin.

Threonine oxidation. Hepatocyte incubations were performed in triplicate, in a total volume of 1 ml of Krebs-Henseleit buffer containing 6–10 mg dry wt of cells, gassed with O2-CO2 (95:5), and incubated at 37°C in 25-ml Erlenmeyer flasks. Hepatocytes were preincubated for 20 min before the addition of substrates. Threonine oxidation was determined by measuring the production of 14CO2 from L-[1-14C]-threonine.
14C]threonine during timed incubations. Unless otherwise indicated, L-[1-14C]threonine was present at 0.5 mM (0.24 MBq/nmol) to provide a physiologically relevant effective concentration within a range observed in plasma, and it was added after the preincubation period. Additional compounds, when provided, were included at the following concentrations: 0.5 mM cysteamine, an inhibitor of the glycine cleavage system (30); 0.5 mM α-cyanoacetamidate (in DMSO), a competitive inhibitor of 2-oxoacid uptake into mitochondria (15); 0.3 mM glycine; 0.3 mM (0.15 MBq/nmol) [1-14C]glycine; and 10−7 M glucagon. When determined, rates of glycine oxidation were measured as described for threonine. Substrate vehicles were added to control incubations to account for the possible impact of vehicle on threonine oxidation. Incubations were terminated at 30 min by the addition of 0.2 ml of 30% (wt/vol) perchloric acid and the 14CO2 was collected into a trapping agent (NCS tissue solubilizer; Amersham, Oakville, ON, Canada) contained in a plastic-center well suspended into the incubation flasks through rubber stoppers. A 60-min collection period was used to ensure the total collection of CO2 (confirmed in previous studies). Center wells were transferred to scintillation vials containing 15 ml of a scintillation cocktail (Omnifluor, Du Pont-New England Nuclear), and total radioactivity was determined by liquid scintillation counting (model 1214 Rackbeta LSC; LKB Wallac, Turku, Finland).

Threonine transport. Threonine transport into isolated rat hepatocytes was measured essentially as described by Salter et al. (28). Briefly, rat hepatocytes were isolated as described in Isolation of rat hepatocytes and preincubated with or without 10−7 M glucagon for 20 min. After the preincubulation period, L-[1-14C]threonine was added to a final concentration of 0.5 mM (0.24 MBq/nmol). At 5, 35, 65, 95, and 125 s after the addition of threonine, 1-ml aliquots were rapidly removed from separate incubation flasks and transferred to 1.5-ml microcentrifuge tubes containing 0.25 ml of a silicone oil mixture (2:1 vt/vol; Dow Corning 550 silicone oil and dinonyl phthalate) layered on top of 0.1 ml of 6% (vol/vol) perchloric acid. The tubes were centrifuged at 18,000 g for 15 s to draw cells through the silicone oil and into the acid layer, leaving the incubation media on top of the oil. Once centrifuged, the tubes were placed at −80°C for 60 min, and then the tubes were cut midway through the silicone oil layer. The bottom acid layer, containing the intracellular L-[1-14C]threonine, was placed in a scintillation vial containing 15 ml of Omnifluor scintillation cocktail, and total radioactivity was determined by liquid scintillation counting. The volume of the extracellular space carried down through the silicone oil was estimated from the values obtained during separate experiments. The effects of dietary protein level, glucagon, and glycine inclusion in the media were determined by three-factor ANOVA examining main effects and interactions. The effects of specific inhibitors on threonine and glycine oxidation rates were evaluated against control values by Dunnett’s procedure (33).

RESULTS

Hepatocytes, isolated from chow-fed rats, oxidized the 1-C of threonine to CO2 at rates of 1.41 nmol·mg dry weight−1·30 min−1 (Table 1). Preincubation of hepatocytes with glucagon resulted in a significant increase (P < 0.05) in threonine oxidation rates. The addition of 0.3 mM glycine to the incubation media did not significantly affect threonine oxidation rates, irrespective of the presence or absence of glucagon in the incubation media. Hepatocytes isolated from rats that had consumed a 60% casein (high-protein) diet had a twofold higher rate of threonine oxidation vs. chow-fed controls. The main effects, from a three-factor ANOVA, of dietary protein level and glucagon exposure were significant, whereas the presence of glycine in the media was not (P < 0.05). There were no significant interactions (Table 1).

Threonine oxidation rates increased as the concentration of threonine in the incubation increased (Fig. 2). Glucagon stimulated threonine oxidation above control levels (P < 0.05) at all threonine concentration levels examined (Fig. 2).

The effects of specific inhibitors on control and glucagon-stimulated rates of threonine and glycine oxidation are presented in Tables 2 and 3 for hepatocytes isolated from chow-fed and 60% casein-fed rats, respectively. Relative to corresponding control values, glucagon stimulated (P < 0.05) both threonine and glycine oxidation rates. The presence of α-cyanoacetamidate, a competitive inhibitor of mitochondrial 2-oxoacid uptake, in the incubation media significantly depressed threonine oxidation rates without affecting the oxidation of l-[1-14C]threonine in hepatocytes

<table>
<thead>
<tr>
<th>Chow Fed (n = 6)</th>
<th>60% Protein Fed (n = 5)</th>
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<tr>
<td>−Glycine + Glycine</td>
<td>−Glycine + Glycine</td>
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<tr>
<td>−Glucagon 1.41 ± 0.41</td>
<td>1.14 ± 0.36</td>
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<tr>
<td>+Glucagon 2.34 ± 0.89</td>
<td>1.70 ± 0.64</td>
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Values are means ± SD and are expressed as nanomoles of 14CO2 produced per milligram of dry weight of hepatocytes per 30 min. Hepatocytes were isolated from rats consuming either standard rodent chow (20% crude protein) or a 60% casein-based diet, incubated in the presence or absence of glycine and glucagon in the medium. Main effects of ANOVA: protein level, P < 0.05; glucagon, P < 0.05; glycine, P > 0.05, not significant. There were no significant interactions.

Materials. The ingredients for the 60% casein diet were obtained from ICN (Cleveland, OH). Collagenase, used in the digestion and isolation of hepatocytes, was purchased from Worthington Biochemicals (Freehold, NJ). L-[1-14C]threonine was purchased from American Radiolabeled Chemicals (St. Louis, MO). [1-14C]glycine and [carboxy-14C]glucagon were purchased from New England Nuclear (Mandel Scientific; Guelph, ON, Canada). Glucagon was purchased from Sigma Chemical (Oakville, ON, Canada). Dinonyl phthalate was purchased from Fisher Scientific (Fairlawn, NJ), and Dow silicone oil was purchased from William F. Nye (New Bedford, MA). All other chemicals were of the highest grade available and, unless otherwise indicated above, were purchased from Sigma.

Statistical analysis. Data are presented as means ± SD from four separate experiments. Mean threonine and glycine oxidation rates are given for control incubations under given conditions. Data for studies involving inhibitors or glucagon are expressed as a percentage of the corresponding control values determined during separate experiments. The effects of dietary protein level, glucagon, and glycine inclusion in the media were determined by three-factor ANOVA examining main effects and interactions. The effects of specific inhibitors on threonine and glycine oxidation rates were evaluated against control values by Dunnett’s procedure (33).
tion of [1-14C]glycine. In addition, α-cyano-cinnamate abolished the stimulatory effect of glucagon on threonine oxidation. The presence of cysteamine, an inhibitor of the mitochondrial glycine cleavage enzyme, reduced glycine oxidation to less than one-quarter of the compounds were: threonine, 0.5 mM; glycine, 0.3 mM; α-cyano-cinnamate, 0.5 mM; cysteamine, 0.5 mM; and glucagon, 10⁻⁷ M.

Table 2. Effect of glucagon, α-cyano-cinnamate, and cysteamine on the production of 14CO₂ from l-[1-14C]threonine and [1-14C]glycine in incubations of hepatocytes isolated from rats fed standard rodent chow

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<tbody>
<tr>
<td></td>
<td>− Glycine²</td>
<td>+ Glycine²</td>
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<tr>
<td>Control¹</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>(1.41 ± 0.41)</td>
<td>(1.14 ± 0.36)</td>
</tr>
<tr>
<td>+ Glucagon²</td>
<td>163 ± 21*</td>
<td>147 ± 14*</td>
</tr>
<tr>
<td>+ α</td>
<td>29 ± 9*</td>
<td>40 ± 6*</td>
</tr>
<tr>
<td>+ Cyanocinnamate²</td>
<td>44 ± 4*</td>
<td>53 ± 8*</td>
</tr>
<tr>
<td>+ Glucagon² + α</td>
<td>82 ± 8</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>+ Cyanocinnamate²</td>
<td>135 ± 6</td>
<td>151 ± 47</td>
</tr>
<tr>
<td>+ Cysteamine²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Glucagon² + Cysteamine²</td>
<td>160 ± 9‡</td>
<td>143 ± 11</td>
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Values are means ± SD and are expressed as a percentage of control values. Absolute oxidation values for the control incubation conditions are presented in parentheses and are expressed as nanomoles of 14CO₂ produced per milligram of dry weight of hepatocytes per 30 min. ¹All incubations were performed in a final volume of 1 ml of Krebs-Henseleit buffer, gassed with 95% O₂-5% CO₂, and incubated for 30 min. ²If added to the media, the final concentrations of the compounds were: threonine, 0.5 mM; glycine, 0.3 mM; α-cyano-cinnamate, 0.5 mM; cysteamine, 0.5 mM; and glucagon, 10⁻⁷ M. ³Significantly different (P < 0.05) from corresponding control (within column) by Dunnett’s procedure.

DISCUSSION

Mammalian cells do not possess the necessary enzymes for the transamination of threonine. The entrance of this indispensable amino acid into catabolic pathways (outlined in Fig. 1) represents its irrevocable loss for the purposes of protein synthesis. Therefore, the identification of factors regulating threonine oxidation is critical to the development of nutritional strategies designed to optimize threonine availability for protein deposition. This is critical not only for the human clinical nutrition setting but for livestock feeding as well. Threonine is typically the second or third limiting amino acid in swine diets based on corn and soybean meal. The movement to reduce crude protein levels in swine diets, due to concerns over excessive nitrogen excretion rates and its potential environmen-
nal impact (18), requires the supplementation of diets with expensive, crystalline sources of the limiting amino acids, including threonine. Minimizing the irrevocable oxidative losses of threonine will help to ensure the optimal delivery of this amino acid for protein synthesis. A better understanding of the factors regulating threonine oxidation will also help in the formulation of clinical dietary strategies to treat patients suffering from wasting diseases. The present studies were designed to investigate factors affecting the hepatic oxidation of threonine.

Except for a small amount of threonine dehydrogenase activity present in the pancreas (21), the catabolic enzymes of threonine metabolism are primarily localized to the liver. We have previously used the rat isolated hepatocyte model system for the investigation of factors affecting the hepatic utilization of specific amino acids, including the oxidation of glycine through the glycine cleavage system (17) and the flux of glutamine through glutaminase (7). In the present studies, we demonstrate that isolated rat hepatocytes have the ability to oxidize \(L-[1^{14}C]\)threonine to \(^{14}CO_2\) and that this production of labeled \(CO_2\) is sensitive to the concentration of threonine in the incubation medium (Fig. 2). The demonstration that the 1-carbon of threonine can be oxidized to \(CO_2\) permitted the examination of the relative importance of specific metabolic pathways to threonine oxidation and the effects that dietary protein level and glucagon exposure have on the irrevocable losses of threonine.

Threonine oxidation can be broadly classified into two distinct routes: glycine-dependent and glycine-independent pathways (Fig. 1). In the glycine-dependent route, the 1-carbon of threonine is lost as \(CO_2\) when glycine, produced from both the threonine dehydrogenase and threonine aldolase pathways, is metabolized by the mitochondrial glycine cleavage enzyme. Therefore, if excess unlabeled glycine is provided in the incubation media, it will dilute any \(L-[1^{14}C]\)glycine coming from the catabolism of threonine, and the total production of \(^{14}CO_2\) will be reduced. However, the coinoculation of hepatocytes with 0.5 mM \(L-[1^{14}C]\)threonine and 0.3 mM glycine did not lead to a significant reduction in \(^{14}CO_2\) production rates relative to the incubations in which additional exogenous glycine was not present. We interpret this lack of effect as signifying that the majority of \(^{14}CO_2\) produced during the metabolism of \(L-[1^{14}C]\)threonine did not arise from the glycine-dependent routes of threonine metabolism.

To further explore the importance of glycine-dependent metabolism to threonine oxidation, hepatocytes were incubated in the presence of cysteamine, a potent inhibitor of the glycine cleavage enzyme (32). An independent assessment of the oxidation of \(L-[1^{14}C]\)glycine in the incubation media confirmed that the inhibitory effect of cysteamine, as \(^{14}CO_2\) production, from hepatocytes isolated from chow-fed rats (Table 2) was reduced to 24% of control values. When cysteamine was included in the incubation medium, the oxidation of \(L-[1^{14}C]\)threonine was not significantly different from control values. Therefore, our results support only a minor role for the glycine-dependent pathways, involving the enzymes threonine dehydrogenase and/or threonine aldolase, in the hepatic oxidation of the 1-carbon of threonine to \(CO_2\).

To directly assess the importance of the glycine-independent pathways, hepatocytes were incubated in the presence of \(\alpha\)-cyanocinnamate, a potent competitive inhibitor of 2-oxobutyrate uptake into mitochondria (15). The demonstration that \(^{14}CO_2\) production was reduced to 29% of control values when \(\alpha\)-cyanocinnamate was included in the incubation medium provides strong evidence that the glycine-independent threonine dehydratase pathway is the predominant pathway in the oxidation of the 1-carbon of threonine. To confirm the specificity of the inhibition, the oxidation of \(L-[1^{14}C]\)glycine was measured in the presence of \(\alpha\)-cyanocinnamate and was found not to differ from control values (Table 2).

When examined in toto, these data provide strong evidence that the glycine-independent pathways of threonine oxidation predominate in hepatocytes freshly isolated from rats fed standard rodent chow. As outlined in Fig. 1, there are two routes whereby the 1-carbon of threonine can be oxidized to \(CO_2\) independently of glycine oxidation: 1) the threonine dehydratase pathway and 2) the spontaneous decarboxylation of 2-amino-3-oxobutyrate, the direct product of the threonine dehydrogenase enzyme. However, Dale (9) has previously shown that there is a tight coupling of threonine dehydrogenase with 2-amino-3-oxobutyrate-CoA ligase. This coupling would effectively channel 2-amino-3-oxobutyrate-CoA toward intramitochondrial glycine formation and acetyl-CoA regeneration rather than spontaneous decarboxylation. As such, our data would support the threonine dehydratase pathway as predominating in the oxidative catabolism of threonine. The substantial inhibition observed in \(^{14}CO_2\) production from \(L-[1^{14}C]\)threonine when hepatocytes were incubated with \(\alpha\)-cyanocinnamate further strengthens the position that threonine dehydratase is responsible for the bulk of threonine oxidized in isolated rat hepatocytes. These present data provide strong evidence that ~65% of threonine oxida-

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**Fig. 3.** Effect of glucagon in the incubation media on threonine transport in isolated rat hepatocytes. *Significantly different (\(P < 0.05\)) from corresponding time point value without glucagon, by \(t\)-test.
tion occurs through the glycine-independent threonine dehydratase pathway in rat hepatocytes.

There is significant variation in the estimates of the relative contributions of the dehydrogenase and the dehydratase pathways to hepatic threonine catabolism. For example, our data are somewhat at odds with those published by Ballèvre et al. (1), who documented an 80% contribution of the dehydrogenase pathway to total threonine oxidation in the pig, and Bird and Nunn (4), from studies conducted with liver homogenates obtained from normally fed rats. On the basis of prevailing intracellular threonine concentrations, the latter authors calculated the relative contribution of the three pathways using Michaelis-Menten kinetics. Their calculations support a major role for the threonine dehydrogenase pathway (87%) in hepatic threonine catabolism in the normally fed rat. Moundras et al. (24) have questioned the assumptions made by Bird and Nunn specifically on the basis that the prevailing intrahepatic threonine concentrations were too low, leading to an underestimation of the contribution of the dehydratase pathway. When using feline hepatocytes, Hammer et al. (16) have shown that the dehydrogenase and dehydratase pathways contribute approximately equal amounts to the oxidation of threonine to CO2. In studies measuring the oxidative fate of [1-13C]threonine, Darling et al. (10) have shown that the dehydrogenase pathway accounts for 44% of total threonine oxidation in infants. Similar studies in adult humans by the same group (11), however, indicate that the dehydrogenase pathway plays a relatively unimportant role (7–11%) in the oxidative catabolism of threonine. It is clear that estimates of the relative contribution of the pathways of threonine catabolism are affected by species differences, stage of maturity, and modeling approaches. A strength of the current study includes the use of highly specific inhibitors of discrete pathways of oxidation, thus enabling a more precise identification of those pathways responsible for the oxidation of threonine.

Hepatocytes isolated from rats consuming high protein (60%) diets oxidized L-[1-14C]threonine to 14CO2 at significantly higher rates compared with hepatocytes isolated from rats consuming standard chow diets (20% protein). However, when the L-[1-14C]threonine oxidative responses to specific inhibitors of the glycine-dependent and -independent pathways are compared between the hepatocytes isolated from chow-fed and the 60% casein-fed rats, a similar pattern is observed. Possible explanations for the similar pattern of responses in the glycine-dependent and glycine-independent oxidative pathways include 1) increased flux through both enzymatic pathways by protein feeding and 2) increased threonine transporter activity.

Studies in rats have documented inductions of threonine dehydratase activity by high-protein feeding but have documented no effect on the activity of threonine dehydrogenase (4). In contrast, high-protein feeding (50% of diet) did induce increased threonine dehydrogenase activity in feline hepatocytes (16) and chick mitochondria (12). The specific signals that regulate increased induction of the dehydratase and dehydrogenase activities by high-protein feeding have not been fully elucidated but may be partially mediated through increased glucagon secretion (13). Hepatic threonine dehydratase activity, but not threonine dehydrogenase activity, has been shown to be increased by a single glucagon injection to rats 4 h before being killed (4). With respect to another glucogenic amino acid, glycine, the incubation of hepatocytes with the hormone glucagon is known to increase glycine oxidation through the mitochondrial glycine cleavage system (17). In the present study, acute exposure of isolated hepatocytes to glucagon resulted in a significant stimulation (~60%) in the production of 14CO2 originating from L-[1-14C]threonine across the range of threonine concentrations tested. The stimulatory effect of glucagon on 14CO2 originating from L-[1-14C]threonine was present with the hepatocytes isolated from the rats consuming the high-protein diets as well.

These data emphasize the impact that both acute and, presumably, chronic glucagon exposure (via high-protein feeding), have on the metabolism of the glucogenic amino acid threonine. The question remains as to the mechanism(s) involved in the increased catabolism observed. An increase in threonine oxidation might generally arise from 1) an increased activity of enzymes via allosteric regulation and/or covalent modification, 2) increased expression of enzymes, and 3) increased delivery of substrate to enzymes. Although induction of enzyme synthesis may provide an explanation for the increases in threonine oxidation observed during chronic glucagon exposure, it is unlikely to account for the increased oxidation observed from hepatocytes in response to an acute exposure to glucagon, due to the short time interval involved. In addition, the enzyme threonine dehydratase does not appear to be regulated by either allosteric mechanisms or covalent modification (29). The fact that the pattern of inhibition observed in the glycine-dependent and -independent pathways was similar between the hepatocytes incubated with and without glucagon, despite an almost twofold increase in the absolute rate of threonine oxidation, suggests an alternate point of regulation. It must be appreciated that, in the current study, threonine oxidation was measured using an intact cell system so that transport mechanisms must be included as a component of the flux. Previous research, in whole animals (6) or perfused liver systems (5), have documented an increased net hepatic uptake of threonine during hyperglucagonemia. In the current study, glucagon increased threonine influx into isolated hepatocytes (Fig. 3), indicating that transporters on the plasma membrane, the mitochondrial membranes, or both may be increased upon the binding of glucagon to its receptor on the plasma membrane, thus providing at least a partial explanation for the increased oxidation of threonine observed in the presence of glucagon. The specific acute mode of action whereby glucagon acts to increase threonine influx is not fully understood but may involve changes mediated through an increase in membrane potential (23). Whether mitochondrial...
amino acid transport is increased due to glucagon stimulation is unknown; however, Mabrouk et al. (22) have shown that glucagon leads to a phosphorylation of cytoplasmic proteins and a concomitant increase in flux through the glycine cleavage system in hepatocytes. The latter results highlight the potential for second messenger systems to mediate an increase in threonine influx into the mitochondria. Ewart et al. (14) have previously shown that glycine transport into the mitochondria is too rapid to account for observed increases in glycine oxidation through the mitochondrial glycine cleavage system upon stimulation by glucagon. Whether the same is true for threonine is unknown.

In the current studies, the impact of glucagon on the oxidation and transport of threonine was determined while hepatocytes were incubated in an amino acid-free Krebs-Henseleit buffer system. Whether the presence of other amino acids at their prevailing in vivo concentrations might have had an impact on the uptake and utilization of threonine was not specifically tested; therefore, comparisons with the situation in vivo must be made with caution. However, it is clear that the capacity for threonine oxidation is proportionally maintained at threonine concentrations 10-fold greater than those routinely used during our incubations (Fig. 2), potentially minimizing the impact of competitive inhibition by other amino acids on threonine metabolism. Future work should include an examination of the impact that other amino acids, including those sharing common transport and metabolic pathways (e.g., serine), have on threonine metabolism in hepatocytes. In addition, the use of $^{13}$C or $^{15}$N isotopomers of threonine may yield new information on the regulation and partition of threonine metabolism through specific enzyme pathways.

In conclusion, our results support the idea that the threonine dehydratase pathway (glycine independent) is the dominant pathway of threonine catabolism in intact rat hepatocytes, accounting for ~65% of the measured threonine oxidation. The production of $^{14}$CO$_2$ through both glycine-dependent and glycine-independent pathways was proportionally increased by prior feeding of high-protein diets or by acute incubation of hepatocytes with glucagon. The observed increases were mediated, at least in part, through an increase in the transport of threonine into hepatocytes.

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