Regulation of the Glycine Cleavage System in the Isolated Perfused Rat Liver*

Robert K. Hampson, Melanie K. Taylor, and Merle S. Olson

From the Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284

(Received for publication, May 16, 1983)

The catabolism of glycine in the isolated perfused rat liver was investigated by measuring the production of $^{14}$CO$_2$ from [1-$^{14}$C]- and [2-$^{14}$C]glycine. Production of $^{14}$CO$_2$ from [1-$^{14}$C]glycine was maximal as the perfusate glycine concentration approached 10 mM and exhibited a maximal activity of 125 nmol of $^{14}$CO$_2$ g$^{-1}$·min$^{-1}$ and an apparent $K_m$ of approximately 2 mM. Production of $^{14}$CO$_2$ from [2-$^{14}$C]glycine was much lower, approaching a maximal activity of approximately 40 nmol of $^{14}$CO$_2$ g$^{-1}$·min$^{-1}$ at a perfusate glycine concentration of 10 mM, with an apparent $K_m$ of approximately 2.5 mM. Washout kinetic experiments with [1-$^{14}$C]glycine exhibited a single half-time of $^{14}$CO$_2$ disappearance, indicating one metabolic pool from which the observed $^{14}$CO$_2$ production is derived. These results indicate that the glycine cleavage system is the predominant catabolic fate of glycine in the perfused rat liver and that production of $^{14}$CO$_2$ from [1-$^{14}$C]glycine is an effective monitor of metabolic flux through this system.

Metabolic flux through the glycine cleavage system in the perfused rat liver was inhibited by processes which lead to reduction of the mitochondrial NAD(H) redox couple. Infusion of $\beta$-hydroxybutyrate or octanoate inhibited $^{14}$CO$_2$ production from [1-$^{14}$C]glycine by 33 and 50%, respectively. Alternatively, infusion of acetoacetate stimulated glycine decarboxylation slightly and completely reversed the inhibition of $^{14}$CO$_2$ production by octanoate. Metabolic conditions which are known to cause a large consumption of mitochondrial NADPH (e.g. ureogenesis from ammonia) stimulated glycine decarboxylation by the perfused rat liver. Infusion of pyruvate and ammonium chloride stimulated production of $^{14}$CO$_2$ from [1-$^{14}$C]glycine more than 2-fold. Lactate plus ammonium chloride was equally as effective in stimulating glycine decarboxylation by the perfused rat liver, while alanine plus ammonium chloride was ineffective in stimulating $^{14}$CO$_2$ production.

There is little information in the literature concerning the mechanism(s) which regulate the rate of glycine catabolism in vivo. The necessity of regulating glycine catabolism is emphasized by the numerous important metabolic fates and functions of glycine in various tissues (1). In addition, there are a variety of clinically defined hyperglycinemic states which display reduced glycine catabolism (2, 3) and which in several cases are secondary consequences of genetic disorders in other metabolic pathways, particularly those involved in the catabolism of the branched chain amino acids (4-7).

The principal pathway by which glycine is catabolized in mammals (8) is the glycine cleavage system. This multicomponent system resides exclusively in the mitochondrial compartment and catalyzes the tetrahydrofolate-dependent cleavage of glycine to yield carbon dioxide, ammonia, N$^5$N$^{10}$-methylene tetrahydrofolate, and one reducing equivalent as NADH + H$^+$ (for review, cf. Ref. 9). The glycine cleavage system is markedly similar in composition and reaction mechanism to the various $\alpha$-keto acid dehydrogenase complexes (10).

In a previous report, we determined that metabolic flux through the glycine cleavage system, in isolated, fully functional rat liver mitochondria was very sensitive to the oxidation/reduction state of both the NAD(H) and NADP(H) redox couples (11). Conditions which lead to oxidation of the mitochondrial pyridine nucleotide redox couples (e.g. state 5, uncouplers, propionate) stimulated flux through the glycine cleavage system while reducing conditions (e.g. respiratory inhibitors, or alternative reducing substrates) inhibited this process. Independent manipulation of the NAD(H) and NADP(H) redox couples demonstrated the sensitivity of glycine decarboxylation by isolated rat liver mitochondria to each redox couple.

The present study was initiated to determine the physiological relevance of our proposal that hepatic glycine catabolism is regulated by the oxidation/reduction state of the mitochondrial NAD(H) and NADP(H) redox couples in an intact cellular system, i.e. the isolated perfused rat liver. It has many times been the case that experimental observations made in one system (i.e. isolated mitochondria) do not apply in more complex systems (i.e. the intact cell and/or organ). Many of the metabolic conditions routinely employed in mitochondrial studies, such as state 3, uncoupled, and respiratory inhibited states, although useful for defining and investigating functional relationships in mitochondria are, in reality, quite nonphysiological, as the metabolic state of the mitochondrion in the intact hepatocyte closely approximates state 4 (12). More importantly, there may exist a cytosolic process(es) which contributes to, and/or influences the regulation of, glycine catabolism. One such possibility is the conversion of glycine to serine by serine hydroxymethyl transferase and subsequent conversion of serine to pyruvate by serine dehydratase, an enzyme present exclusively in the cytosol (13). Metabolism of pyruvate by the pyruvate dehydrogenase complex will result in the production of carbon dioxide originating from the carboxyl position of glycine.

The results of the present study indicate that the glycine cleavage system is responsible almost entirely for the production of $^{14}$CO$_2$ from [1-$^{14}$C]glycine by the isolated perfused rat liver.

* This research was supported by Grant AM-19473 from the National Institutes of Health and Grant AQ-728 from the Robert A. Welch Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
liver and that metabolic flux through the glycine cleavage system is highly sensitive to the oxidation/reduction state of the mitochondrial NAD(H) and NADP(H) redox couples.

MATERIALS AND METHODS

Male, Sprague-Dawley rats (180-200 g body weight) were fed a standard laboratory chow and water ad libitum and anesthetized prior to surgery with sodium pentobarbital (10 mg/100 g body weight, intraperitoneally). Livers were perfused in situ, employing a hemoglobin-free, nonrecirculating perfusion technique (14) and a flow rate of 35 ml·min⁻¹ was established. The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (15), pH 7.4, saturated with a mixture of oxygen/carbon dioxide (95%/5%) maintained at 37 °C. Substrates were infused into the perfusion system immediately prior to the liver as indicated by the horizontal bars in the individual figures. Oxygen consumption by the liver was monitored continuously using a Clark-type oxygen electrode placed in the perfusion circuit immediately following the liver. Infusion of substrates was not initiated until after a steady state level of oxygen consumption by the liver was obtained. Samples of the effluent perfusate were collected at 30-s intervals and production of 14CO₂ was measured in 5-ml portions of the effluent perfusate, as described previously (11). By measuring the specific radioactivity of the infused [1-¹⁴C]glycine, a continuous measurement of the metabolic flux through the glycine cleavage system was obtained.

Radiolabeled [1-¹⁴C]- and [2-¹⁴C]glycine were purchased from Research Products International. Liquid scintillation counting scintillation fluid was purchased from National Diagnostics and phenylisothiocyanate from Fisher. Acetoacetate and lactate were purchased from Sigma; alanine and β-hydroxybutyrate were purchased from Calbiocal-Behring; and pyruvate was obtained from Boehringer Mannheim. All other reagents were of the highest grade available from commercial suppliers.

RESULTS

We have demonstrated, in isolated rat liver mitochondria, that metabolic flux through the glycine cleavage system is highly sensitive to the oxidation-reduction state of the mitochondrial NAD(H) and NADP(H) redox couples (11). To investigate the question of whether or not these mechanisms can be operative under conditions as near to in vivo as possible, the catabolism of glycine, by the glycine cleavage system, was investigated in the isolated perfused rat liver. As with the studies in isolated rat liver mitochondria, the metabolic flux through the glycine cleavage system was monitored by measuring the production of 14CO₂ from [1-¹⁴C]glycine. In order to assess the validity of employing this procedure to monitor flux through the glycine cleavage system in the perfused rat liver, the experiments depicted in Figs. 1 and 2 were performed.

The production of 14CO₂ from [1-¹⁴C]- and [2-¹⁴C]glycine, by the perfused rat liver was measured as a function of the infused glycine concentration (Fig. 1). Decarboxylation of [1-¹⁴C]glycine by the perfused liver was near maximal as the glycine concentration in the perfusate approached 10 mM, with a measured activity of approximately 125 nmol·g⁻¹ wet weight·min⁻¹ and an apparent Kₚ of 2.5 mM. Production of 14CO₂ from [2-¹⁴C]glycine approached a maximal rate of 10 nmol·g⁻¹·min⁻¹ at a glycine concentration of 10 mM, with an apparent Kₚ of approximately 2.5 mM. These observed rates of 14CO₂ production from [1-¹⁴C]- and [2-¹⁴C]glycine by the perfused liver are in the appropriate range, based on estimates of mitochondrial protein/g wet weight in rat liver (16) and our previous studies of the glycine cleavage system in isolated rat liver mitochondria (11), in which the glycine cleavage system was demonstrated to be responsible for the observed 14CO₂ production.

Illustrated in Fig. 2 is an experiment in which the liver was perfused with unlabeled glycine for 30 min prior to the addition of a tracer amount of [1-¹⁴C]glycine for a 35-min interval. The production of 14CO₂ was measured throughout the period of tracer infusion. Following termination of [1-¹⁴C]glycine infusion, "washout" or the "off" kinetics of 14CO₂ production indicated a single half-time of label disappearance with t₁/₂ = 7.5 min. In a steady state of 14CO₂ production from [1-¹⁴C]glycine, each individual process leading to production of 14CO₂ will be operating at a particular rate, with the sum of these rates equating the observed steady state rate of 14CO₂ production. Upon termination of the tracer [1-¹⁴C]glycine infusion, the label will disappear from the metabolic pool of glycine supplying each 14CO₂ producing process, at a rate dependent on the rate of each 14CO₂ producing process and with a first order rate constant. The observation of a single kinetic component of 14CO₂ production, in Fig. 2, indicates there is likely one metabolic pool from which the observed production of 14CO₂ from [1-¹⁴C]glycine is...
derived. This type of experimental approach has been employed previously with a variety of other substrates (e.g., pyruvate, branched chain α-keto acids, and glucose) (17–19) in studying various decarboxylation processes. The longer half-time for 14CO2 washout observed with [1-14C]glycine as compared with these other substrates (e.g., 7.5 min versus approximately 15 s to 1 min) likely reflects a high intraacellular glycine concentration. Measured concentrations of glycine in liver tissue are among the highest of all the free amino acids (20, 21) with estimates ranging from 5 to 14 mM (after correction for extracellular space). Only one kinetic component of 14CO2 disappearance was observed at all concentrations of perfusate glycine examined from 0.5 to 5 mM, and in all cases nearly identical half-times of 14CO2 disappearance were observed. The efficiency of glycine clearance during a single pass through the liver was observed to be approximately 2% at 1 mM glycine (data not shown).

Based on the experiments presented in Figs. 1 and 2, it appears that the glycine cleavage system is the predominant catabolic fate of glycine and that measuring the production of 14CO2 from [1-14C]glycine is an effective method for monitoring metabolic flux through the glycine cleavage system in the isolated perfused rat liver.

Catabolism of glycine by the perfused rat liver was investigated under various conditions which lead to either an oxidation or reduction of the mitochondrial respiratory chain. Depicted in Fig. 3 is the effect of acetocacetate, 5 mM, and β-dl-hydroxybutyrate, 10 mM, infusion on glycine decarboxylation by the perfused rat liver. Acetocacetate and β-hydroxybutyrate are interconverted by the mitochondrial enzyme β-hydroxybutyrate dehydrogenase, oxidize and reduce the mitochondrial NAD(H) redox couple, respectively, and are not metabolized further in liver tissue to any appreciable extent. Glycine decarboxylation by the perfused liver was inhibited by 33% upon infusion of β-dl-hydroxybutyrate, 10 mM, consistent with an oxidation-reduction state-mediated effect. The absence of or only minimal stimulation of glycine decarboxylation by acetocacetate infusion likely reflects the fact that the mitochondrial NAD(H) redox couple is already quite oxidized (17, 22, 23) in the absence of a strongly reducing substrate and thus acetocacetate infusion evokes little change in the NAD(H) redox couple under these conditions. A slight stimulation (6–10%) of glycine decarboxylation was observed if the concentration of infused acetocacetate was increased to 20 mM (data not shown). Glycine decarboxylation by the perfused rat liver also was inhibited (50–60%) by infusion of the medium chain fatty acid, octanoate (data not shown), which in the perfused rat liver is known to lead to a reduction of the mitochondrial NAD(H) redox couple (17). The inhibition of glycine decarboxylation by infusion of octanoate was reversed completely by infusion of acetocacetate. Hence, metabolic flux through the glycine cleavage system in the isolated perfused rat liver is sensitive to the oxidation-reduction state of the mitochondrial NAD(H) couple.

One of the major NADPH utilizing processes of liver tissue is the synthesis of urea from ammonia (24). A key enzyme in this process is glutamate dehydrogenase which catalyzes the reductive amination of α-ketoglutarate to synthesize glutamate. The metabolic pathway for the synthesis of urea requires two separate nitrogen donors, ammonia for carbamyl phosphate synthesis and aspartate for synthesis of arginosuccinate. The glutamate synthesized in the glutamate dehydrogenase reaction is utilized to synthesize the aspartate required for urea synthesis. Glutamate dehydrogenase, which is located exclusively in the mitochondrion, can utilize either NADH or NADPH. Chamalaun and Tager (25) and Sies et al. (26) both demonstrated that during synthesis of urea from ammonia by the perfused rat liver there occurs substantial oxidation of the NAD(P)H redox couple. In addition, for this system to operate, there must be an adequate supply of oxaloacetate (e.g., pyruvate via pyruvate carboxylase) and α-ketoglutarate (e.g., pyruvate via the tricarboxylic acid cycle). Krebs et al. (27) have demonstrated that maximal rates of urea synthesis are observed only when there is a source of oxaloacetate and α-ketoglutarate, such as pyruvate, available.

Depicted in Figs. 4 and 5 is the effect of ammonium chloride and pyruvate infusion on decarboxylation of [1-14C]glycine in the perfused rat liver. Fig. 4 illustrates the effect of increasing concentrations of ammonium chloride on glycine decarboxylation in the absence and in the presence of infused pyruvate, 2.5 mM. In the absence of infused pyruvate, infusion of increasing concentrations of ammonium chloride had little or no effect on [1-14C]glycine decarboxylation. If ammonium chloride was infused initially at 10 mM, a somewhat variable but transient stimulation of [1-14C]glycine decarboxylation was observed (data not shown). However, in the presence of infused pyruvate, 2.5 mM, there occurred a substantial stimulation of glycine decarboxylation to a rate more than twice the basal rate, with maximal rates observed at approximately
1 mM ammonium chloride. The inhibition of 14CO₂ production at higher concentrations of ammonium chloride may be the result of feedback inhibition of the glycine cleavage system, as ammonia is a product of this reversible reaction. Although there is some inhibition of 14CO₂ production at higher concentrations of ammonium chloride, 10 mM was employed in subsequent experiments to insure maximal rates of NADPH utilization. Depicted in Fig. 5 is the effect of increasing concentrations of infused pyruvate on glycine decarboxylation by the perfused rat liver in the presence of infused ammonium chloride, 10 mM. Maximal rates of glycine decarboxylation were observed with concentrations of pyruvate above 1 mM and again attained a rate more than double the basal rate of 14CO₂ production.

Performance of a washout kinetic experiment in the presence of pyruvate, 2.5 mM, and ammonium chloride, 2.5 mM, exhibited one kinetic component \( t_{1/2} = 7 \text{ min} \), indicating that the observed stimulation of \(^{14}\text{CO}_2\) production was due to a stimulation of glycine cleavage activity and not to other processes (data not shown).

The stimulation of glycine decarboxylation in the perfused liver upon addition of pyruvate and ammonium chloride likely is due to an oxidation of the NAD(P)H redox couple and not the NAD(H) couple, as no stimulation of glycine decarboxylation is observed when acetacetate is infused with pyruvate. Depicted in Fig. 6 is the effect of increasing concentrations of acetacetate in the presence of infused pyruvate, 2.5 mM, on \(^{14}\text{CO}_2\) production. Acetacetate merely relieves the partial inhibition of glycine decarboxylation observed with pyruvate, indicating that the effect of ammonium chloride infusion was not due to an oxidation of the NAD(H) redox couple.

The possibility exists that a cytosolic oxidizing substrate (i.e., pyruvate via the lactate dehydrogenase) may be necessary to observe the stimulation of glycine decarboxylation by the liver. However, the possibility is unlikely as the cytosolic NAD(H) redox couple is already highly oxidized (22, 28, 29) and, furthermore, no stimulation of glycine decarboxylation was observed with infusion of pyruvate and acetacetate (Fig. 6). If oxidation of the cytosolic NAD(H) redox couple were necessary, one should not observe stimulation of glycine decarboxylation with infused lactate and ammonium chloride as lactate is certainly a reducing substrate for the cytosolic NAD(H) redox couple. Depicted in Fig. 7 is the effect of increasing concentrations of infused lactate on glycine decarboxylation by the perfused rat liver in the presence of infused ammonium chloride, 10 mM. Lactate plus ammonium chloride is equally as effective as pyruvate plus ammonium chloride in stimulating production of \(^{14}\text{CO}_2\) from \(^{1-14}\text{C}\)glycine. Infusion of lactate, 5 mM, alone inhibits glycine decarboxylation by about 20% (data not shown). Moreover, stimulation of glycine decarboxylation by pyruvate and ammonium chloride is not abrogated by infusion of ethanol, 2 mM (data not shown). Ethanol is a very potent reducing substrate of the cytosolic NAD(H) redox couple (via alcohol dehydrogenase) and should maintain the cytosolic NAD(H) redox couple in a more reduced state even in the presence of infused pyruvate.

Another important source of pyruvate in liver tissue is alanine, via transamination with \( \alpha \)-ketoglutarate by the glutamatepyruvate transaminase. However, infusion of alanine with ammonium chloride should not stimulate glycine decarboxylation in the perfused rat liver. Transamination of alanine converts \( \alpha \)-ketoglutarate to glutamate, which would tend to drive the glutamate dehydrogenase reaction in the opposite direction, towards oxidative deamination of glutamate. Alternatively, the glutamate formed upon infusion of ammonium
chloride, by increasing the glutamate/α-ketoglutarate ratio, may prevent the transamination of alanine so that little pyruvate is supplied. In either case, no stimulation of glycine decarboxylation by the perfused rat liver should be observed upon infusion of alanine in the presence of infused ammonium chloride and this was found to be the case. Depicted in Fig. 8 is the effect of increasing concentrations of alanine on glycine decarboxylation by the perfused rat liver in the presence of infused ammonium chloride, 10 mM. Infusion of alanine, 5 mM, in the absence of ammonium chloride inhibited glycine decarboxylation by 40% (data not shown).

Based on the evidence supplied by the above experiments, it appears that glycine decarboxylation by the isolated perfused rat liver is sensitive to the oxidation-reduction state of the mitochondrial respiratory chain including both the NAD(H) and NADP(H) redox couples.

**DISCUSSION**

In our previous studies, we reported that glycine catabolism by isolated fully functional rat liver mitochondria was highly sensitive to the oxidation-reduction state of the mitochondrial respiratory chain, including both the NAD(H) and NADP(H) redox couples (11). The primary focus of the present study was to investigate the mechanisms which regulate hepatic glycine catabolism in a system as near as possible to that obtained in vivo, i.e. the isolated perfused rat liver. Experiments illustrated in this report indicate that metabolic flux through the glycine cleavage system, the principle pathway of hepatic glycine catabolism, can be monitored effectively and continuously in the isolated perfused rat liver by measuring the production of $^{14}$CO$_2$ from [1-$^{14}$C]glycine. This contention is supported by the observation of appropriate rates of $^{14}$CO$_2$ production from [1-$^{14}$C]- and [2-$^{14}$C]glycine, based on our previous studies of the glycine cleavage system in isolated rat liver mitochondria in which glycine cleavage was demonstrated to be responsible for the observed production of $^{14}$CO$_2$. Further support for the glycine cleavage system being the predominant fate of infused glycine was the appearance of a single $t_{1/2}$ of $^{14}$CO$_2$ disappearance in washout kinetic experiments, indicating a single metabolic pool from which the observed production of $^{14}$CO$_2$ from [1-$^{14}$C]glycine is derived.

Metabolic flux through the glycine cleavage system in the isolated perfused rat liver was sensitive to the oxidation-reduction state of the mitochondrial redox couples. Conditions which led to the oxidation of the mitochondrial respiratory chain stimulated glycine decarboxylation, while reducing conditions inhibited this process.

The observed sensitivity of metabolic flux through the glycine cleavage system in the isolated perfused rat liver to the oxidation-reduction state of the mitochondrial respiratory chain was expected. First, we demonstrated previously the sensitivity of the glycine cleavage system to the oxidation-reduction state in isolated rat liver mitochondria. Additionally, one of the four components of the glycine cleavage system is a dihydrolipoyl dehydrogenase (30), quite possibly identical to the dihydrolipoyl dehydrogenase component of the α-keto acid dehydrogenase complexes (e.g. pyruvate, α-ketoglutarate, and branched chain α-keto acid). The regulatory effects of the oxidation-reduction state of the mitochondrial respiratory chain on these enzyme complexes in the isolated perfused rat liver are well established (17, 18, 31) and are directionally consistent with the observed effects on the glycine cleavage system.

Measurements of the tissue levels of the pyridine nucleotides also were consistent with the results described above (data not shown). However, the significance of whole tissue levels of nucleotides and/or metabolites, when one is concerned only with the mitochondrial compartment, is debatable.

Several of our results indicated that metabolic flux through the glycine cleavage system in the perfused rat liver is sensitive to the oxidation-reduction state of the NADP(H) redox couple as well as the NADP(H) redox couple. Conditions which are known to lead to a large consumption of mitochondrial NADPH, i.e. ureogenesis from ammonia (24–27), greatly stimulated decarboxylation of glycine by the perfused rat liver. Infusion of ammonia and either pyruvate or lactate stimulated glycine decarboxylation more than 2-fold. This stimulation of glycine decarboxylation likely was due to an oxidation of the NADP(H) redox couple and not the NAD(H) couple as there was no stimulation observed when ammonium chloride was replaced with acetocetate.

The mechanism by which the NADP(H) oxidation-reduction couple may regulate flux through the glycine cleavage system is not immediately obvious. The mechanism likely is not expressed in the same fashion as is the NAD(H) redox effects. First, there is no precedence for NADP(H) effects on dihydrolipoyl dehydrogenase and secondly, there is no published evidence that NADP$^+$ is a substrate for the glycine cleavage system. In addition, as discussed in our report on the regulation of the glycine cleavage system in isolated mitochondria (11), the observed sensitivity to the NADP(H) redox couple likely is not due to increased metabolism of N$^9$,N$^{10}$-methylene tetrahydrofolate to CO$_2$ and tetrahydrofolate by methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, and formyltetrahydrofolate oxidoreductase. Preliminary results indicate that this is the case in the perfused liver system as well. An investigation is currently in progress to determine the precise mechanism by which the NADP(H) oxidation-reduction couple exerts its regulatory influence on the glycine cleavage system.

The physiological significance of the observed sensitivity of hepatic glycine catabolism to the oxidation-reduction state of the NAD(H) and NADP(H) redox couples can only be speculated upon at this time. Changes in the oxidation-reduction state of these couples in the livers of fed, fasted, or diabetic rats (22) and during ischemia (23, 32) or anoxia (26) have been well documented (for review, see Ref. 33), and therefore one would expect glycine catabolism to change in these various conditions. However, the directional changes in glycine catabolism, the magnitude of such changes, and the physiological utility of these changes in the activity of the glycine
cleavage system in several metabolic states are not immediately obvious and are central points in our experimental consideration of this multienzyme system.

REFERENCES
Regulation of the glycine cleavage system in the isolated perfused rat liver.
R K Hampson, M K Taylor and M S Olson


Access the most updated version of this article at http://www.jbc.org/content/259/2/1180

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/259/2/1180.full.html#ref-list-1