Regulation of the hepatic glycine-cleavage system

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The primary catabolic fate of glycine in mammals involves the hepatic glycine-cleavage system in which glycine is sequentially converted to $\text{CO}_2$, $\text{NH}_3$, $\text{NADH} + \text{H}^+$, and $\text{N}^5,\text{N}^{\text{a}}$-methylene tetrahydrofolate (Fig. 1) (for review, see Kikuchi, 1973). This multi-enzyme reaction is located exclusively in the mitochondrial compartment (Motokawa & Kikuchi, 1971) and can be compared in several respects with the more common 2-oxo acid dehydrogenase multi-enzyme complexes (i.e. the pyruvate, branched-chain 2-oxo acid and 2-oxoglutarate dehydrogenases). While little is known about the regulation of hepatic glycine catabolism, the importance of understanding this process derives from observations that a variety of inherited genetic disorders are accompanied by pronounced hyperglycinaemia (e.g. concentrations of glycine 10–20 times normal in body fluids). Hyperglycinaemic states can be classified 'non-ketotic' (Nyhan, 1978) or 'ketotic' (Tanaka, 1975), and reflect primary defects in the glycine-cleavage system and secondary effects on glycine catabolism caused by disorders in other metabolic pathways (e.g. branched-chain amino, propionic or methylmalonic acid metabolism), respectively.

Recent results from our laboratory have indicated that the metabolic flux through the hepatic glycine-cleavage reaction can be monitored effectively both in isolated mitochondrial systems (Hampson et al., 1983, 1984a) and in the isolated perfused rat liver (Hampson et al., 1984b) by measuring the production of $[^1]\text{C} \text{CO}_2$ from $[^1]\text{C}$glycine. In isolated rat liver mitochondria metabolic conditions which result in the oxidation of the NAD(H) and NADP(H) oxidation-reduction couples caused rapid rates of $[^1]\text{C}$glycine decarboxylation (e.g. State 3 or uncouplers) while reducing conditions (e.g. respiratory chain inhibitors or strongly reducing substrates in State 4) led to inhibited rates of $[^1]\text{C}$glycine decarboxylation. While manipulation of mitochondrial adenine nucleotide ratios per se did not correlate with the rate of glycine cleavage, changes in the aforementioned NAD(H) and NADP(H) oxidation-reduction couples exhibited very close correlations with the measured metabolic flux through the glycine-cleavage reaction.

Short-chain-length fatty acids such as propionate caused a marked acceleration of glycine cleavage in rat liver
mitochondria in the absence of a strongly reducing substrate because of the acceleration of NADP(H) oxidation by the mitochondrial energy-linked transhydrogenase (Hampson et al., 1984a).

Glycine cleavage in the isolated perfused rat liver was investigated with a similar experimental rationale as in the mitochondrial studies discussed above (Hampson et al., 1984b). Maximal rates of 1-14C-glycine decarboxylation (e.g. 125 nmol/min per g) were attained at perfusate glycine concentrations approaching 10 mm. Infusion of metabolic substrates such as 3-hydroxybutyrate or octanoate inhibited the rate of glycine cleavage by 33 and 50%, respectively. Metabolic conditions (e.g. NH4Cl, 10 mm, plus pyruvate or lactate, 5 mm) which have been demonstrated to cause rapid consumption of intramitochondrial NADPH (Chamalaun & Tager, 1970; Siess et al., 1975) during urea synthesis caused an approximately three-fold increase in glycine decarboxylation by the perfused rat liver.

The stimulated rate of glycine decarboxylation and the synthesis of urea were inhibited nearly completely by propionate. It has been suggested that propionyl-CoA and/or medium-chain fatty acids inhibit selected enzymic reactions in urea synthesis (Gruskay & Rosenberg, 1979; Coude et al., 1979; Martin-Requrero et al., 1983) and it is likely that, in the present study, propionate inhibited urea synthesis, thus preventing the consumption of intramitochondrial NADPH which in turn inhibited the glycine-cleavage reaction. Several laboratories have investigated the inhibitory effects of cysteamine on the glycine-cleavage reaction (e.g. see Yudkoff et al., 1984; Hayasaka & Tada, 1983). In the present study the rapid rates of glycine decarboxylation observed under ureogenic conditions were inhibited nearly completely by co-infusion of cysteamine. The precise mechanism by which cysteamine inhibits glycine decarboxylation is presently under investigation.

In summary, our experimental approach has demonstrated that the hepatic glycine-cleavage system is regulated primarily by the oxidation–reduction state of the intramitochondrial NAD(H) and NADP(H) couples. Whether this type of regulation is physiologically important under normal metabolic conditions and/or contributes to pathological hyperglycinaemic states, remains to be demonstrated definitively.

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Regulation of branched-chain amino acid metabolism

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A high-capacity system for branched-chain amino acid catabolism is needed to prevent the apparent toxic effects of these compounds and their metabolites seen in the human clinical condition known as maple syrup urine disease. On the other hand, catabolism must be tightly controlled, particularly when dietary protein intake is restricted, to assure continuous availability of these essential amino acids for protein synthesis. A large number of physiological conditions (e.g. starvation, diabetes, trauma) influence branched-chain amino acid metabolism, attesting to the importance of the regulatory mechanisms associated with their catabolism. Mechanisms that regulate blood levels of branched-chain amino acids may also be of considerable physiological importance, since one of these amino acids (leucine) inhibits protein degradation, stimulates protein synthesis and promotes insulin release. The possible therapeutic value of supplemental dietary branched-chain amino acids or their 2-oxo acid analogues for the treatment of various disease states (e.g. muscular dystrophy, hepatic encephalopathy) is receiving attention.

Regulation of branched-chain amino acid catabolism is achieved in large part by the branched-chain 2-oxo acid dehydrogenase complex. This enzyme is responsible for the oxidative decarboxylation of all three 2-oxo acids derived by transamination of the branched-chain amino acids. As with pyruvate dehydrogenase, the branched-chain 2-oxo acid dehydrogenase is an intramitochondrial multi-enzyme complex subject to regulation by covalent modification (Fatania et al., 1981; Odessey, 1982; Paxton & Harris, 1982). Specificity of branched-chain 2-oxo acid dehydrogenase for 2-oxo acids is much less than that of pyruvate dehydrogenase, suggesting that branched-chain 2-oxo acid dehydrogenase may function not only in the catabolism of branched-chain amino acids but also in the catabolism of threonine and methionine (Paxton et al., 1986a) and perhaps even pyruvate (Goodwin et al., 1986). The branched-chain 2-oxo acid dehydrogenase kinase, believed to be distinct from pyruvate dehydrogenase kinase, is an integral component of the branched-chain 2-oxo acid dehydrogenase complex and is inhibited by branched-chain 2-oxo acids (Lau et al., 1982; Paxton & Harris, 1984a), 2-chloro-4-methylpentanoate (Harris et al., 1982a), phenylpyruvate (Paxton & Harris, 1984b), clofibrate (Paxton & Harris, 1984b) and di-