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Increased activity of renal glycine-cleavage-enzyme complex in metabolic acidosis

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Glycine is metabolized in isolated renal cortical tubules to stochiometric qualitities of ammonia, CO_2 and serine by the combined actions of the glycine-cleavage-enzyme complex and serine hydroxymethyltransferase. The rate of renal glycine metabolism by this route is increased in tubules from acidotic rats, but is not affected *in vitro* by decreasing the incubation pH from 7.4 to 7.1. Metabolic acidosis caused an increase in the renal activity of the glycine-cleavage-enzyme complex, but there were no changes in the activity of serine hydroxymethyltransferase or of methylenetetrahydrofolate dehydrogenase. This enzymic adaptation permits increased ammoniagenesis from glycine during acidosis. The physiological implications are discussed.

INTRODUCTION

The mechanisms whereby the mammalian kidney increases its production of ammonia during metabolic acidosis have been the object of intense study during the past two decades. Since the principal source of urinary ammonia is glutamine (Pitts, 1972), much work has focused on the regulation of the metabolism of this amino acid in kidney cells. Two different levels of metabolic regulation have been uncovered. At one level, which operates rapidly, the existing metabolic machinery is modulated. An example of this is the acute activation of glutamine hydrolysis occasioned by a fall in pH and mediated via direct effects on 2-oxoglutarate dehydrogenase (Lowry & Ross, 1980). At a second level there are slower, adaptive, responses in which the amounts of individual enzyme proteins are altered. The best established example of this phenomenon is the increased activity of renal glutaminase in chronically acidotic rats (Rector *et al.*, 1955).

Glutamine is not, however, the sole source of urinary ammonia. Other amino acids, in particular glycine, are significant sources. There is an appreciable removal of glycine from the circulation by the kidneys of man, dog and rat (Owen & Robinson, 1963; Shalhoub *et al.*, 1976; Squires *et al.*, 1976). Pitts *et al.* (1965) have shown, by experiments with ^{15}N-labelled glycine in dogs, that glycine can contribute about 5% of renal ammonia. We have reported that kidneys of acidotic rats remove twice as much glycine from the circulation as do kidneys from normal rats (Lowry *et al.*, 1985). The basis for this increased renal extraction of glycine in acidosis is not known, but it could be attributed either to increased substrate supply (glycine concentrations increase in acidosis) or to an increased renal capacity to metabolize glycine. That the latter may occur is suggested by the studies of Davies & Yudkin (1952), with kidney slices, and Pitts (1971), with the perfused kidney. Both of these studies demonstrated that kidneys from acidotic rats could produce more ammonia from glycine than do kidneys from control rats. The present study was designed to investigate this problem, with a view to identifying the step(s) responsible for increased renal glycine metabolism in acidosis.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats of 350–450 g were used for all experiments. They were allowed free access to food and water at all times. Acidosis was induced by replacing water with 0.38 M-NH_4Cl for 7 days.

Tissue preparations

Isolated cortical tubules were prepared from the renal cortex by collagenase digestion (Guder *et al.*, 1971). Tubules were incubated with 5 mM-glycine at a tubule concentration of 2–5 mg dry wt./ml in 25 ml polypropylene flasks containing 2 ml of Krebs–Henseleit (1932) buffer and were equilibrated with O_2/CO_2 (19:1). For the experiments described in Table 3, the pH of the medium was decreased by adding sufficient 1 M-HCl to lower the bicarbonate concentration by 15 mM.

Incubations were terminated after 30 min by the addition of $HClO_4$ (final concn. 3%, v/v). The precipitated protein was removed by centrifugation (5000 g, 10 min) and the supernatants were neutralized. In some experiments $^{14}CO_2$ produced from [1-^{14}C]- and [2-^{14}C]-glycine (52.9 and 47.3 Ci/mol respectively; NEN Canada, Lachine, Quebec, Canada) was trapped in hanging centre wells (Kontes, Vineland, NJ, U.S.A.) containing 0.2 ml of NCS tissue solubilizer (NEN Canada) and counted for radioactivity in Omnifluor (NEN Canada). Ammonia was assayed enzymically (Kirsten *et al.*, 1963) and serine by amino acid analysis (Brosnan *et al.*, 1983).

Mitochondria were prepared from the renal cortex of normal and acidotic rats as described for liver by Hampson *et al.* (1983). They were resuspended at a final protein concentration of 20 mg/ml. The supernatant from the first centrifugation at 6500 g for 20 min was used for the assay of cytoplasmic enzymes.

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Enzyme assays

Serine hydroxymethyltransferase (EC 2.1.2.1) and methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5) were assayed by the methods of Taylor & Weissbach (1965) and Tan *et al.* (1977) respectively.

The glycine-cleavage complex (EC 2.1.2.10) was assayed as the production of $^{14}CO_2$ from [1-^{14}C]glycine by mitochondria incubated in a hypo-osmotic medium. The assay medium contained, in a final volume of 1 ml: 25 μmol of Tris/HCl, pH 8.0, 10 μmol of dithiothreitol, 250 nmol of pyridoxal 5′-phosphate, 500 nmol of NAD^+, 500 nmol of tetrahydrofolate prepared as described by Snell (1980), and 10 μmol of [1-^{14}C]glycine (0.5 μCi). The assay was started by the addition of 1 mg of mitochondrial protein. After 10 min incubation at 37 °C, the assay was terminated by the addition of 0.1 ml of $HClO_4$ (30%, v/v), and $^{14}CO_2$ was collected into 0.2 ml of NCS tissue solubilizer placed in hanging centre wells. All assays were linear with respect to both protein concentration and time of incubation under the conditions described.

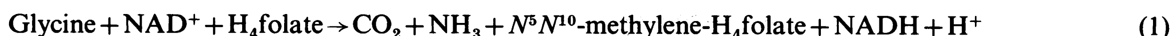
Protein was determined by the biuret procedure (Gornall *et al.*, 1949), with bovine serum albumin as standard.

Calculations and statistics

For the determination of V_{max} and K_m values for glycine-cleavage enzyme, the non-linear-regression program described by Greco *et al.* (1982) was used. All values are shown as means ± S.D. for the numbers of separate experiments indicated in the Table legends. Statistical significance was determined by the use of Student's t test for unpaired data.

RESULTS AND DISCUSSION

Glycine is metabolized in rat kidney by the combined action of the glycine-cleavage-enzyme complex (eqn. 1) and serine hydroxymethyltransferase (eqn. 2):



$$\text{Glycine} + NAD^+ + H_4\text{folate} \rightarrow CO_2 + NH_3 + N^5N^{10}\text{-methylene-}H_4\text{folate} + NADH + H^+ \quad (1)$$

$$N^5N^{10}\text{-Methylene-}H_4\text{folate} + \text{glycine} \rightarrow \text{serine} + H_4\text{folate} \quad (2)$$

such that the overall conversion is given by eqn. (3):

$$2\ \text{Glycine} + NAD^+ \rightarrow CO_2 + NH_3 + NADH + H^+ + \text{serine} \quad (3)$$

Table 1. Effect of chronic acidosis on glycine metabolism by isolated renal cortical tubules

Experimental details are described in the text. $^*P < 0.05$, $^{**}P < 0.01$, for differences between tubules from normal and acidotic rats.

	Measurement (μmol/h per g dry wt.)	
Status of rats ...	Normal ($n = 7$)	Acidotic ($n = 6$)
Net serine synthesis	54.2 ± 10.3	90.3 ± 17.7**
Net ammonia production	63 ± 31	96 ± 33
[1-^{14}C]Glycine conversion into $^{14}CO_2$	63.2 ± 14.0	109.4 ± 12.1*
[2-^{14}C]Glycine conversion into $^{14}CO_2$	8.9 ± 1.7	10.2 ± 2.7

The experiments described in Table 1 show the stoichiometric production of serine, ammonia and $^{14}CO_2$ from [1-^{14}C]glycine by kidney cortex tubules. Furthermore, there is a 50–70% increase in glycine metabolism in kidney tubules from acidotic rats. The rate of conversion of [2-^{14}C]glycine into $^{14}CO_2$ was low, consistent with the fact that this carbon atom is not extensively oxidized in renal tissue but is extensively incorporated into serine.

To determine whether acute acidosis can stimulate ammoniagenesis from glycine, incubations were carried out at pH 7.4 and 7.1 with glycine as sole substrate. The decreased incubation pH did not affect the rate of conversion of glycine into ammonia, serine or CO_2 (Table 2). This contrasts with the situation with glutamine as substrate, where ammoniagenesis is stimulated by a similarly decreased incubation pH (Lowry & Ross, 1980).

To establish the enzymic basis for the increased rate of renal glycine metabolism observed in kidney tubules from acidotic rats (Table 1), we measured the activities of several enzymes that could be important for this phenomenon. The glycine-cleavage complex (eqn. 1) catalyses the initial reaction of glycine catabolism in the kidney. Serine hydroxymethyltransferase (eqn. 2) exists in both the cytoplasm and the mitochondria (Nakamo *et al.*, 1968), and catalyses the synthesis of serine from glycine and methylenetetrahydrofolate. Methylenetetrahydrofolate dehydrogenase, which exists in both the cytoplasm and the mitochondria (Yoshida & Kikuchi, 1972), catalyses the first reaction in the sequence that could oxidize methylenetetrahydrofolate, and hence C-2 of glycine to CO_2. The data in Table 3 demonstrate a significant increase in the activity of the glycine-cleavage complex in mitochondria from acidotic animals. There was no change in the activity of serine hydroxymethyltransferase, or of methylenetetrahydrofolate dehydrogenase. The extremely low activity of this enzyme in mitochondria is consistent with the low rate of oxidation of C-2 of glycine to CO_2 (Table 1). Since production of methylenetetrahydrofolate by the action of the glycine-cleavage complex occurs only in mitochondria, the oxidation of this C_1 moiety to CO_2 would require a substantial activity of methylenetetrahydrofolate dehydrogenase in mitochondria. The low activity of this enzyme in mitochondria would tend to favour the metabolism of mitochondrial methylenetetrahydrofolate via serine hydroxymethyltransferase, and hence the formation of serine. Thus the enzyme activities reported here are consistent with the stoichiometric production from glycine of CO_2, NH_3 and serine reported in Table 1. Such stoichiometry has also been observed by Rowsell *et al.* (1982).

The increased glycine-cleavage activity observed in acidosis (Table 3) could be due to an increased quantity of the enzyme protein or to some activation of the enzyme. Such an activation, if it occurs, would be

Table 2. Effect of decreased incubation pH on glycine metabolism by isolated renal cortical tubules

Experimental details are described in the text.

	Measurement (μmol/h per g dry wt.)	
pH...	7.4 ($n = 4$)	7.1 ($n = 4$)
Net serine synthesis	55.8±9.3	57.2±14.3
Net ammonia production	70.3±20.9	68.8±24.7
[1-^{14}C]Glycine conversion into $^{14}CO_2$	64.5±12.4	66.9±16.7
[2-^{14}C]Glycine conversion into $^{14}CO_2$	8.7±2.1	7.3±1.4

Table 3. Effect of metabolic acidosis on the activity of glycine-metabolizing enzymes in the rat kidney

Experimental details are described in the text. The values in parentheses express enzyme activity as nmol/min per g of kidney. *$P < 0.05$ for differences between normal and acidotic rats.

		Activity (nmol/min per mg of protein)	
Enzyme	n	Normal	Acidotic
Glycine-cleavage complex			
Mitochondrial	4	2.70±0.49 (148.5)	4.06±0.31* (223.3)
Serine hydroxymethyl-transferase			
Cytosolic	3	5.67±1.56 (544.3)	5.43±1.35 (521.3)
Mitochondrial	3	6.58±1.51 (361.9)	7.91±1.43 (435.1)
Methylenetetrahydrofolate dehydrogenase			
Cytosolic	3	8.1±2.3 (777.6)	8.7±1.7 (835.2)
Mitochondrial	3	0.62±0.14 (34.1)	0.41±0.25 (22.6)

expected to alter the kinetic characteristics of the enzyme. We therefore examined the activity of the glycine-cleavage complex as a function of glycine concentration. Plots of velocity against substrate concentration yielded rectangular hyperbolas for enzyme from both normal and acidotic animals (results not shown). Calculation of the K_m and $V_{max.}$ showed increased activity in acidotic animals ($V_{max.}$ 5.5±1.6 and 11.9±2.5 nmol/min per mg of protein; $n = 3$, $P < 0.05$), with no alteration in K_m (8.1±1.8 and 8.3±0.9 mM respectively). The lack of an alteration in the K_m for glycine in acidosis is consistent with the possibility that the increased enzyme activity is due to an increased quantity of enzyme protein, but is not definitive in this matter. The activities of glycine-cleavage enzyme found in these experiments are somewhat higher than those in Table 3. This is because the activities measured in these latter experiments are $V_{max.}$ activities, whereas those in Table 3 were obtained at glycine concentrations (10 mM) that were not saturating. The enzyme activities reported above correspond to activities of 80 and 170 μmol/h per g dry wt. of kidney in the normal and acidotic animals respectively (assuming 60 mg of mitochondrial protein/g and a wet-wt./dry-wt. ratio of 4). This compares with fluxes through the enzyme in isolated tubules of 63 and 109 μmol/h per g dry wt. (Table 1). The proposition that the increased activity of glycine-cleavage enzyme evident in acidosis is responsible for the increased production of NH_3, CO_2 and serine implies that the glycine-cleavage enzyme, not serine hydroxymethyltransferase, is rate-limiting for the overall metabolic transformation.

A number of enzymic adaptations have been well established in the acidotic rat kidney. Principal among these are the increased activities of glutaminase (Rector *et al.*, 1955), glutamate dehydrogenase (Seyama *et al.*, 1973) and phosphoenolpyruvate carboxykinase (Alleyne, 1970). The first two adaptations permit increased deamidation of glutamine and increased deamination of the glutamate so produced. The increased activity of phosphoenolpyruvate carboxykinase permits increased conversion of glutamine carbon into glucose. Pitts (1971) has also demonstrated an increased activity of alanine aminotransferase in the acidotic rat kidney. None of these adaptations can facilitate increased ammoniagenesis from glycine, however.

The physiological importance of ammoniagenesis from glycine is of interest. Glutamine is by far the most important ammoniagenic precursor, whereas glycine normally only contributes about 5% of urinary ammonia. The question arises as to why the kidney requires a second ammoniagenic precursor and whether it becomes quantitatively more important in certain situations. The renal metabolism of glycine via glycine-cleavage enzyme and serine hydroxymethyltransferase produces serine, which may be converted into glucose in the liver. However, gluconeogenesis from glycine would be equally served if the entire transformation occurred in the liver. Hannaford *et al.* (1982) have shown that, in starvation, muscle proteolysis in man is driven not only by the need to supply amino acids for hepatic gluconeogenesis but also by the need to supply glutamine for renal ammoniagenesis. In fact they demonstrated that provision of $NaHCO_3$, to correct the acidosis of starvation, lessens the loss of lean body mass. Thus it would be physiologically advantageous for the kidney to use, as ammoniagenic precursors, amino acids released by muscle in addition to glutamine. During starvation, glycine, as well as alanine and glutamine, is one of the major amino acids released by muscle in man (Felig *et al.*, 1973) and rat (Ishikawa, 1976).

It has also been demonstrated that the renal uptake of glycine increases about 5-fold during prolonged starvation in man, whereas the splanchnic extraction of this amino acid ceases (Felig *et al.*, 1969). The shift of glycine catabolism from liver to kidney may therefore serve to spare somewhat the renal requirement for glutamine and thus decrease the loss of lean body mass. Felig *et al.* (1969) observed that the increased renal glycine extraction

observed in the prolonged-starved man could not be due solely to increased plasma concentrations of glycine, and postulated a primary intrarenal stimulation of glycine metabolism. The present studies demonstrate such an effect in kidneys of metabolically acidotic rats. We postulate that the increased glycine-cleavage activity observed by us in kidneys of acidotic rats is an important physiological adaptation that permits increased ammoniagenesis from an amino acid other than glutamine.

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