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EFFECT OF pH ON METABOLISM OF THE GLUTAMINE CARBON SKELETON BY RENAL CORTICAL MITOCHONDRIA

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Abstract—1. To determine the effect of altered acid–base homeostasis on the intramitochondrial metabolism of the glutamine carbon skeleton $^{14}CO_2$ production from [U-^{14}C]glutamine by isolated rat renal cortical mitochondria was measured.

2. Mitochondria from rats with chronic metabolic acidosis either showed no change or diminished $^{14}CO_2$ production in comparison with pair fed controls.

3. By contrast, when the pH of the medium incubating mitochondria from normal rats was manipulated (pH 7.0, 7.4, 7.7), $^{14}CO_2$ production was clearly altered, but the direction and magnitude of the change depended on the glutamine concentration used (0.5 or 10.0 mM).

4. Mitochondria produced significant quantities of $^{14}CO_2$ when [1,4 ^{14}C]succinate was used as substrate, indicating that $^{14}CO_2$ production from glutamine does not originate solely from the decarboxylation of α KG.

5. Thus chronic acidosis and pH, per se, affect intramitochondrial glutamine carbon skeleton metabolism in different fashions, but the specific mechanism cannot be elucidated using $^{14}CO_2$ production from [U-^{14}C]glutamine.

6. Additional studies directly quantitating the metabolic products of glutamine have confirmed these findings and more precisely defined the sites of metabolic alteration.

INTRODUCTION

Conversion of the carbon skeleton of glutamine to glucose by rat renal cortex is accelerated in chronic metabolic acidosis, presumably due to an increase in activity of the rate limiting cytoplasmic enzyme phosphoenolpyruvate carboxykinase (Alleyne & Scullard, 1969; Flores & Alleyne, 1971). Incubation of renal tissue at low pH also stimulates glucose formation, but the mechanism is unknown (Goodman *et al.*, 1966; Alleyne *et al.*, 1973; Irias & Greenberg, 1972; Kamm *et al.*, 1967; Nagata & Rasmussen, 1970; Preuss, 1971). The direct influence, if any, of acid–base perturbations on the intramitochondrial metabolism of the carbon skeleton of glutamine is unclear. In one previous report $^{14}CO_2$ formation from [U-^{14}C]glutamine by isolated renal cortical mitochondria was unaltered by metabolic acidosis but stimulated by incubation at a low pH (Simpson & Sherrard, 1969). However, in studies from another laboratory a decrease in $^{14}CO_2$ formation at a low pH was observed (Kurokawa & Rasmussen, 1973). In view of these conflicting results and observations with intact renal tissue indicating that tracer methodology might lead to spurious conclusions (Vinay *et al.*, 1978), we reinvestigated this issue.

MATERIALS AND METHODS

Renal cortical mitochondria isolated from male Sprague–Dawley rats were incubated as described previously (Tannen & Kunin, 1976a,b; Kunin & Tannen, 1979a] with 0.5 to 10 mM L-[U-^{14}C]glutamine (New England Nuclear Corporation). In some studies, tracer amounts of [1,4-^{14}C]succinate (New England Nuclear Corporation) were added to flasks containing 0.5 mM succinate. After incubation for 10–20 min, 0.3 ml of Hyamine hydroxide, 10× (Packard) was injected through the serum stoppers into a suspended plastic center well, followed immediately by the rapid injection of 1 ml of 1 M perchloric acid into the incubation mixtures to stop the reaction and release CO_2. Shaking was resumed for 30 min to allow complete diffusion of $^{14}CO_2$ into the Hyamine. The plastic center wells containing Hyamine were then transferred into counting vials, 10 ml of a toluene phosphor system added and radioactivity assayed with a Packard Tricarb Liquid Scintillation Spectrometer. The phosphor system contained 0.3% 2,5-diphenyloxazole and 0.01% p-bis (5-phenyloxozolyl-2) benzene in toluene. Counting efficiency was consistently between 80–82%. Mitochondrial protein was determined by the biuret reaction.

Studies utilizing intact renal cortical mitochondria isolated from rats with chronic metabolic acidosis or potassium deficiency, as well as incubations with mitochondria from normal rats at different media pHs (7.0, 7.4 and 7.7, achieved by equimolar manipulation of bicarbonate and chloride concentration), were carried out in paired fashion as described previously (Tannen & Kunin, 1976a,b).

These data are reported as nmol $CO_2 \cdot min^{-1} \cdot mg^{-1}$ mitochondrial protein. Statistics were performed using paired or non-paired Student's *t*-test.

RESULTS

Preliminary studies confirmed that $^{14}CO_2$ production was linear during the period of incubation chosen.

Table 1. Effect of chronic metabolic acidosis or potassium depletion on mitochondrial $^{14}CO_2$ production from [U-^{14}C]glutamine

	1.0 mM Gln		10.0 mM Gln	
	nmol $CO_2/mg^{-1} \cdot min^{-1}$			
	Normal	Acidotic	Normal	Acidotic
Mean	37.4	32.4	48.1	36.1
SE	+3.3	±1.7	±4.4	±3.5
n	7		7	
P	NS		<0.01	
	Normal	K^+ Dep.	Normal	K^+ Dep.
Mean	31.6	30.4	37.6	32.6
SE	±1.2	±2.3	±2.7	±2.4
n	7		7	
P	NS		<0.05	

The effect of either chronic metabolic acidosis or potassium deficiency on $^{14}CO_2$ formation from glutamine is shown in Table 1. With both conditions $^{14}CO_2$ production is unchanged with 1.0 mM glutamine as substrate and significantly decreased in comparison with pair fed controls when 10 mM glutamine is used.

The results of experiments in which mitochondria from normal rats were incubated at three different medium pHs are shown in Table 2. The results are dependent on the concentration of media glutamine used. With 0.5 mM glutamine $^{14}CO_2$ production from [U-^{14}C]glutamine is lowest at pH 7.7; while with 10 mM glutamine $^{14}CO_2$ production is lowest at pH 7.0.

To determine whether $^{14}CO_2$ formation from [U-^{14}C]glutamine resulted solely from decarboxylation of alpha ketoglutarate to succinate, mitochondria from normal animals were incubated at pH 7.4 with 0.5 mM, 1,4-^{14}C-labeled succinate. Preliminary studies indicated that $^{14}CO_2$ production was negligible with 2,3-^{14}C-labeled succinate. In seven studies $^{14}CO_2$ production from [1,4 ^{14}C]succinate averaged 6.0 ± 0.8 nmol.min^{-1}.mg^{-1}. Thus a significant fraction of the $^{14}CO_2$ produced during incubations with glutamine originates from substrate decarboxylation subsequent to succinate, in spite of the absence of any 2 carbon donor added to the incubation medium.

In a series of separate studies performed under identical conditions intramitochondrial metabolism of alpha ketoglutarate was quantitated by measuring the key nitrogen and carbon metabolites of glutamine (Kunin & Tannen, 1979a,b). A comparison of these data with the experiments measuring $^{14}CO_2$ generation yielded reasonable similarity.

Table 2. Effect of alterations in medium pH on mitochondrial $^{14}CO_2$ production from [U-^{14}C]glutamine

	0.5 mM Gln			10 mM Gln		
pH	7.0	7.4	7.7	7.0	7.4	7.7
Mean	27.8	28.5	20.1	29.0	37.1	41.9
SE	±2.1	±2.5	±1.8	±2.0	±2.0	±2.3
n	6	6	6	10	9	8
P	NS	<0.01		<0.01	NS	

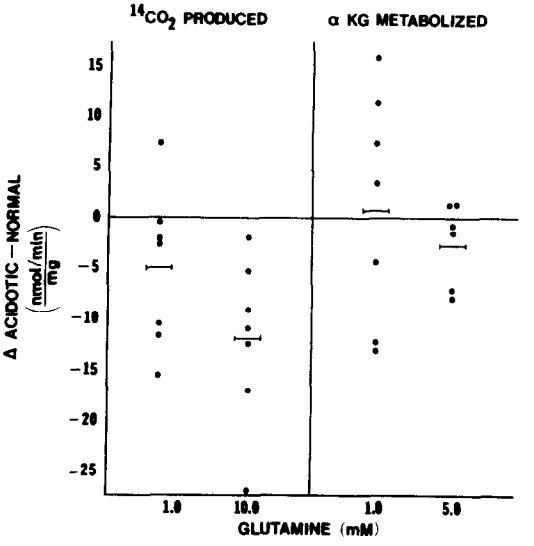

Fig. 1. Effect of chronic acidosis on metabolism of the glutamine carbon skeleton. Each point represents a comparison between mitochondria from a rat with chronic metabolic acidosis and a pair fed control, and the horizontal bar the mean change for the entire group. In this and subsequent figures, the left panel depicts changes in $^{14}CO_2$ production from [U-^{14}C]glutamine, and the right panel changes in directly quantitated αKG metabolized. $^{14}CO_2$ production is unchanged by acidosis at 1.0 mM glutamine and decreased significantly ($P < 0.01$) at 10 mM. αKG metabolized is unchanged at both substrate concentrations.

As shown in Fig. 1, αKG metabolism was unaltered by chronic acidosis using both low and high concentrations of glutamine; $^{14}CO_2$ production was not significantly changed at 1 mM glutamine and was decreased with higher levels of substrate.

Manipulations of pH *in vitro* are shown in Figs 2 and 3. A high pH significantly depresses both $^{14}CO_2$ production and calculated αKG metabolized at low glutamine concentrations, but not at higher substrate levels (i.e. 5 or 10 mM) (Fig. 2). A low pH significantly diminishes both $^{14}CO_2$ production and αKG metabolized at high substrate concentrations, and significantly stimulates αKG metabolized ($P < 0.05$) at 0.5 mM glutamine (Fig. 3). Thus the pattern of response is similar with direct measurement of αKG metabolized and with an indirect assessment utilizing $^{14}CO_2$ production. Although the isotopic technique does not provide a quantitative measure of the decarboxylation of αKG to succinate, it does appear to qualitatively reflect changes in this intramitochondrial process.

DISCUSSION

Since renal ammoniagenesis can be influenced by the metabolism of the glutamine carbon skeleton, metabolic regulation of this process could play an important role in modulating ammonia production (Tannen, 1978). In chronic acidosis the increase in PEPCK activity directly alters metabolism of the carbon products of glutamine in the cytoplasm and may indirectly influence intramitochondrial events. Whether acidosis, or pH per se, directly modifies the

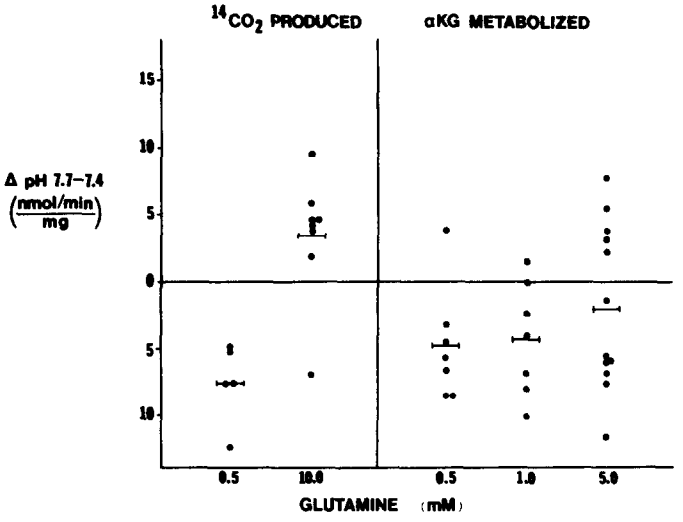

Fig. 2. Effect on an alkaline pH on metabolism of the glutamine carbon skeleton. The data represent a comparison between normal mitochondrial incubated at pH 7.7 and 7.4. $^{14}CO_2$ production is diminished significantly ($P < 0.01$) at 0.5 mM glutamine but unchanged at 10 mM. αKG metabolized is decreased significantly at 0.5 mM ($P < 0.05$) and 1.0 mM ($P < 0.05$), and unchanged at 5 mM.

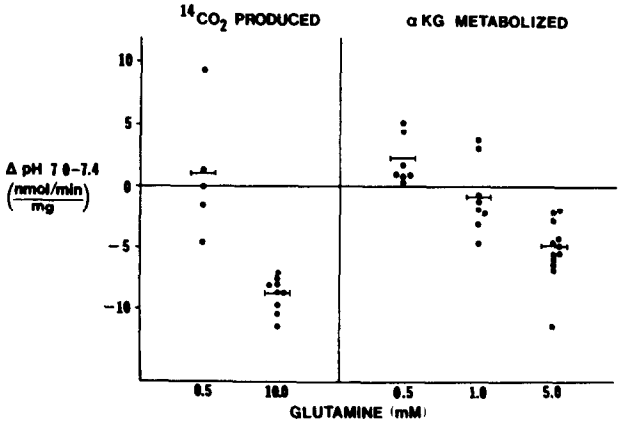

Fig. 3. Effect of an acid pH on metabolism of the glutamine carbon skeleton. The data represent a comparison between normal mitochondria incubated at pH 7.0 and 7.4. $^{14}CO_2$ production is unchanged at 0.5 mM glutamine and decreased significantly ($P < 0.01$) at 10 mM. αKG metabolized is increased significantly at 0.5 mM ($P < 0.05$), unchanged at 1.0 mM, and decreased significantly ($P < 0.001$) at 5 mM glutamine.

intramitochondrial metabolism of the glutamine carbon skeleton is undefined.

Consistent with the prior observation of Simpson & Sherrard (1969) using dogs, our studies of $^{14}CO_2$ generation from [U-^{14}C]glutamine by rat kidney mitochondria suggest that chronic acidosis either has no effect or diminishes intramitochondrial metabolism of the glutamine carbon skeleton. By contrast, pH manipulations of the medium incubating normal mitochondria indicate that pH, *per se*, directly affects mitochondrial glutamine carbon metabolism. The response of $^{14}CO_2$ production to pH manipulations *in vitro* is highly dependent on the glutamine concentration employed, which appears to account for the conflicting findings reported by previous investigators. Simpson and Sherrard, utilizing 0.5 mM glutamine found more $^{14}CO_2$ produced at lower media pHs (Simpson & Sherrard, 1969); while Kurokawa & Rasmussen (1973), utilizing 2 mM glutamine, found a 17% decrease in $^{14}CO_2$ production at a media pH of 7.0 compared with 7.4. Our results, which indicate that a low pH stimulates $^{14}CO_2$ production with low glutamine concentrations but inhibits at high substrate levels, appear to resolve the discrepancy between these earlier reports.

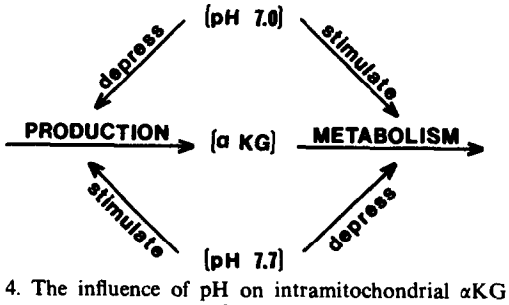

Fig. 4. The influence of pH on intramitochondrial αKG metabolism.

Under the conditions utilized in our experiments, the mitochondria produce significant quantities of $^{14}CO_2$ from labeled succinate, indicating that isotopic CO_2 generation from uniformly labeled glutamine does not solely reflect the decarboxylation of αKG to succinate. With this complexity, as well as the substrate dependence of the response, it is impossible to interpret these observations with $^{14}CO_2$ production in a mechanistic fashion. Nevertheless, they do suggest that chronic acidosis and pH, per se, affect intramitochondrial glutamine carbon skeleton metabolism in a different fashion.

A comparison with studies from our laboratory that assess carbon skeleton metabolism more directly, confirms the qualitative implications of the findings with $^{14}CO_2$ production and clarifies the mechanism responsible for the confusing substrate dependence of the pH response.

In a series of separate studies performed under identical conditions the intramitochondrial metabolism of αKG was quantitated by measuring the key nitrogen and carbon metabolites of glutamine (Kunin & Tannen, 1979a,b). The findings with this approach were reasonably similar to those using $^{14}CO_2$ generation. Both the direct assessment of αKG metabolism as well as the measurement of $^{14}CO_2$ production indicate that chronic acidosis does not directly stimulate the intramitochondrial metabolism of αKG. On the other hand, when pH is manipulated *in vitro*, significant changes are observed with both techniques, but, as shown in Figs 2 and 3, the response is highly dependent on the glutamine concentration used. An acid incubation medium stimulates αKG metabolism at low glutamine concentrations and diminishes it at high substrate levels; while the response to an alkaline pH is the converse, with inhibition at a low glutamine concentrations and no significant change at higher glutamine levels.

Since these studies are carried out with glutamine as substrate the net amount of αKG converted to succinate is dependent on all the factors that influence this enzymatic process, including the amount of substrate available for metabolism. Previous experiments from our laboratory have shown that pH affects the production of αKG from glutamine by isolated mitochondria (Kunin & Tannen, 1979a). When the effect of pH on αKG production and metabolism are considered simultaneously, the data indicate that a low pH depresses production but stimulates the subsequent metabolism of αKG; while a high pH acts in the opposite fashion (Fig. 4). The overall response at a given glutamine concentration depends on which effect predominates, but it seems clear that a low pH favors while a high pH inhibits the intramitochondrial conversion of αKG to succinate.

Thus pH, *per se*, can alter the intramitochondrial metabolism of the glutamine carbon skeleton while chronic acidosis appears to directly modify the cytoplasmic portion of the metabolic pathway. These different sites of action may provide increased flexibility for the regulation of ammonia production during alterations in acid–base homeostasis.

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SYMPOSIUM DISCUSSION

Schoolwerth: We have obtained somewhat different results from experiments in which glutamine alone or glutamine plus physiological concentrations of glutamate were used. When mitochondria were incubated with 1 mM [U-^{14}C]glutamine plus 2 mM cold glutamate, α-ketoglutarate production, measured indirectly by the same method you described, was similar in control and acidosis. However, the specific activity of α-ketoglutarate was two-fold

higher in acidosis compared to control. These findings indicate an alteration in the intramitochondrial metabolism of the carbon skeletons of both glutamine and glutamate in acidosis.

Tannen: Our observations focus on metabolism of the α-ketoglutarate formed. There is no reason to suspect that the metabolism of α-ketoglutarate is influenced by its source.

Vinay: Are your results compatible with the increase in glutaminase activity in acidosis?

Tannen: Yes, in acidotic rats. Addition of acid to isolated mitochondria does not evoke the response that is seen in tubules or in isolated perfused kidney. Thus, part of the response may require the presence of cytosol, or even the whole organ, but I don't think that is the whole answer.

Alleyne: How soon do you see the effects of pH on CO_2 production? The fall in α-ketoglutarate concentration in response to acidosis is very rapid, within minutes (see Lowry, discussion to paper by Vinay *et al.*, p. 97, this volume.

Tannen: We see a fall in intramitochondrial 2-oxoglutarate within 2 minutes in response to acidosis in isolated mitochondria, but have not measured the CO_2 production as early as that.

Alleyne: Is the reduction of intracellular pH which Dr Radda reports (p. 277, this volume) in acidosis, 0.2–0.3 pH units, sufficient to explain the fall in 2-oxoglutarate concentration that you see?

Schoolwerth: We find a ΔpH of about 0.4 units between the medium and mitochondrial matrix.

Vinay: How do you explain the persistently low 2-oxoglutarate concentration in kidneys from chronic acidotic rats, in which the pH is almost back to normal?

Alleyne: The key word there is "almost".

Weiner: Since your incubations are carried out without added ADP, could some of the changes be due simply to an effect of pH on respiratory rate?

Tannen: Dr Simpson, who has looked at this, never saw changes in respiratory rate in response to change in pH, except at very low pH's.

Schoolwerth: I agree that it is not necessary to assume that the same factors which initiate response to acidosis are those which maintain it.

Tannen: Dr Brosnan reported an increase in CO_2 production from [1-^{14}C]glutamine in acidotic mitochondria, in contrast to our own studies. The major difference in technique was that Dr Brosnan's study was performed at room temperature, and in the presence of ADP rather than ATP, as in our study. The kinetics of the metabolic pathway may differ at 37°C and room temperature.

Baverel: What do you see as the role of aspartate in regulation of ammonia production?

Tannen: The role of the transaminase reaction in the intact cell is controversial. The purine nucleotide cycle is one of the possible alternatives.