The relationship between glutamate deamination and gluconeogenesis in kidney

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The effect of 3-mercaptopycolinate, an inhibitor of phosphoenolpyruvate carboxykinase [GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32], was tested on NH_3 formation via the purine nucleotide cycle and glutamate dehydrogenase (EC 1.4.1.2). NH_3 excretion in rats increased 70-fold after 48 h of NH_4Cl feeding, from 12.2 ± 4.5 to $862 \pm 190 \mu\text{mol/mg}$ of creatinine. At 4 h after a single intraperitoneal injection of 3-mercaptopycolinate into NH_4Cl -fed rats, NH_3 excretion was inhibited by 93%. Kidneys of NH_4Cl -fed plus 3-mercaptopycolinate-treated rats, compared with those of NH_4Cl -fed rats, showed a 3.5-fold increase in the content of IMP, 5-fold increase in adenylosuccinate, 4-fold increase in aspartate, and a 30% increase in AMP. 3-Mercaptopycolinate completely inhibited NH_3 and glucose formation from glutamate in tubules from acidotic rats and NH_3 formation from aspartate in kidney perfusion experiments. When transamination in tubules was prevented by 2-amino-4-methoxy-*trans*-but-3-enoic acid, formation of glucose, but not of NH_3 , from glutamate was inhibited. 3-Mercaptopycolinate completely inhibited NH_3 formation from aspartate in the presence of the aminotransferase inhibitor in kidney tubules. The data show that NH_3 can be formed via glutamate dehydrogenase and the purine nucleotide cycle at significant and approximately equal rates. 3-Mercaptopycolinate has no direct effect on NH_3 formation via glutamate dehydrogenase, but inhibits that via the purine nucleotide cycle. We conclude that gluconeogenesis is not regulatory for NH_3 formation in kidney.

Metabolic acidosis in the rat results in an increase in formation of NH_3 and glucose by the kidney, but the relationship between these processes is uncertain. One theory postulates that PEPCK, a rate-limiting step for gluconeogenesis, may be important in augmenting ammoniogenesis (Alleyne & Scullard, 1969). Support for this theory has been provided by experiments using 3-MPA, an inhibitor of PEPCK (DiTullio *et al.*, 1974; Kostos *et al.*, 1975; Blackshear *et al.*, 1975; Goodman, 1975; Robinson & Oei, 1975). Bennett & Alleyne (1976) and Ross (1976) showed that 3-MPA will inhibit formation of glucose and NH_3 from glutamine in

acidotic rat kidney. They concluded that 3-MPA exerted its effect on NH_3 formation by decreasing the rate of glutamate deamination. Therefore PEPCK may regulate NH_3 -forming pathways in kidney. The present study was undertaken using 3-MPA and a transaminase inhibitor, MVG, to examine the pathways of glutamate metabolism in kidney.

Methods

Male Sprague-Dawley rats (approx. 250 g) were obtained from Charles River Breeding Laboratories, Wilmington, MA, U.S.A. They were fed on Purina rat chow *ad libitum* and were given 0.25 M-sucrose to drink. After a control period of 2 days, 1.5% (w/v) NH_4Cl was added to their sucrose drinking solution for 2 days. On the morning of study, the rats were sequestered in metabolic cages for 4 h of urine collection. Some NH_4Cl -fed rats received a single intraperitoneal injection of 3-MPA (75 mg/kg

Abbreviations used: PEPCK, phosphoenolpyruvate carboxykinase [GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32]; 3-MPA, 3-mercaptopycolinate; MVG, 2-amino-4-methoxy-*trans*-but-3-enoic acid.

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body wt.) at the start of the urine-collection period. Urine was collected for 4 h, the animals were then killed, and left kidneys from control, NH_4Cl -fed and NH_4Cl -fed plus 3-MPA-treated rats were rapidly frozen for tissue metabolite analyses as previously described (Bogusky *et al.*, 1981). Other animals were killed and their kidneys removed for tubule-suspension experiments by the method of Guder (1979). Protein was determined by the method of Lowry *et al.* (1951), and ATP, ADP, AMP, 2-oxoglutarate, aspartate, malate, glutamine, NH_3 and glutamate were measured by standard spectrophotometric methods (Bergmeyer, 1974). Urinary ammonia was determined by the method of Seligson & Seligson (1951) and urinary creatinine by the method of Folin (1914). Bovine albumin (fraction V, reagent grade) was obtained from Miles Laboratories, Elkhart, IN, U.S.A. All biochemicals were obtained from Sigma Chemical Corp., St. Louis, MO, U.S.A. Dr. Phillip Miller of Hoffman-LaRoche, Nutley, NJ, U.S.A., kindly provided MVG; 3-MPA was obtained from Smith, Kline and French Laboratories, Philadelphia, PA, U.S.A.

Isolated kidney perfusions were performed on acidotic and non-acidotic rats. Perfusate was prepared as described by Ross *et al.* (1973) and contained 5 mM-glucose. The surgical procedure of Nishiitsutsuji-Uwo *et al.* (1967) was used except for cannulation of the inferior vena cava. Ureters were cannulated with PE₁₀ tubing and urine was allowed to drain freely into the perfusate reservoir. On the day before study, animals used for perfusion or tubule-suspension experiments were given a single intraperitoneal injection of DL-methionine sulfoximine (200 mg/kg body wt.). Results were analysed for statistical significance by Student's *t* test.

Results and discussion

After 48 h of acid feeding, urinary NH_3 excretion in the rats rose from a mean of 12.2 ± 4.5 (S.E.M.) to $862 \pm 190 \mu\text{mol/mg}$ of creatinine. At 4 h after a single injection of 3-MPA, NH_3 excretion fell to $60 \pm 5.7 \mu\text{mol/mg}$ of creatinine.

Renal metabolites

After 48 h of acid feeding, renal IMP content rose 5-fold and adenylosuccinate fell by 50% (Table 1). After both acid feeding and 3-MPA injection, renal contents of IMP rose 15-fold, of adenylosuccinate 2-fold, of aspartate 4-fold, and of AMP 2-fold. The tissue contents of glutamine and malate in these rats increased, whereas glutamate content remained unchanged compared with acid-fed rats not receiving 3-MPA. Glutamine, glutamate and 2-oxoglutarate contents of the kidneys decreased in acid-fed rats.

Table 1. *Effect of 48 h NH_4Cl feeding and 3-mercaptopicolinate on kidney tissue metabolites*

Experimental details are given in the Methods section. Each value is the mean $\pm$ S.E.M. for six experiments. Significance values for 48 h in NH_4Cl -fed rats are versus control rats, and those for 3-MPA + 48 h NH_4Cl -fed rats are versus 48 h NH_4Cl -fed rats. (* $P < 0.050$; † $P < 0.020$; ‡ $P < 0.005$; § $P < 0.001$)

Treatment	Renal metabolites ($\mu\text{mol/g}$ of kidney)		
	Control	48 h NH_4Cl	3-MPA + 48 h NH_4Cl
Glutamine	1.61 ± 0.20	$0.41 \pm 0.08^\dagger$	$0.94 \pm 0.20^*$
Glutamate	3.95 ± 0.12	$2.85 \pm 0.32^*$	2.79 ± 0.20
Aspartate	0.71 ± 0.14	0.65 ± 0.05	$2.78 \pm 0.24^*$
2-Oxo-glutarate	0.52 ± 0.10	$0.30 \pm 0.04^\S$	$0.09 \pm 0.03^\ddagger$
ATP	1.45 ± 0.11	$1.91 \pm 0.11^\dagger$	1.56 ± 0.75
ADP	0.74 ± 0.09	$1.27 \pm 0.08^\ddagger$	1.21 ± 0.05
AMP	0.47 ± 0.09	0.60 ± 0.07	$0.88 \pm 0.07^\dagger$
Malate	0.53 ± 0.05	0.32 ± 0.02	$1.38 \pm 0.20^\S$
(nmol/g of kidney)			
IMP	4.5 ± 2.2	$20.4 \pm 3.7^\ddagger$	$71.1 \pm 14^\S$
Adenylo-succinate	4.5 ± 1.8	2.0 ± 0.6	$10.0 \pm 0.8^\S$

Ammonia formation in perfused kidney

Mean perfusion pressure to the kidney was 110 ± 5 mmHg, with flow rates of 39 ± 2 ml/min. Inulin clearances were 0.65 ± 0.07 ml/min, with a urine flow rate of 0.063 ml/min. Renal sodium reabsorption during the perfusion was greater than 94%. Kidneys perfused with 2 mM-aspartate formed NH_3 at a rate of $1.0 \mu\text{mol/min}$ per g of kidney (Fig. 1, broken lines). Non-acidotic kidney perfused with 2 mM-aspartate contained 12.5 ± 1.5 nmol ($n = 4$) of IMP/g of kidney, which is $2\frac{1}{2}$ times that *in vivo*. When 3-MPA was added to the perfusate, NH_3 production stopped; this was associated with a decrease in the uptake of aspartate, but no change in perfusate glutamine or glutamate contents (Fig. 1, solid lines). The effect of 3-MPA was exactly the same whether the kidneys were from acidotic or non-acidotic rats. In non-acidotic kidneys perfused with 2 mM-glutamine or 2 mM-glutamate, 3-MPA had no effect on NH_3 formation (results not shown), whereas in acidotic kidneys NH_3 formation from these substrates was inhibited by 3-MPA. These observations confirm those of others, that 3-MPA has an effect on NH_3 formation from glutamine and glutamate in acidotic kidneys, but not in kidneys from normal animals (Bennett & Alleyne, 1976; Ross, 1976). However, we observed that 3-MPA can inhibit NH_3 formation from aspartate in both acidotic and non-acidotic kidneys. Perfusion experi-

Fig. 1. Effect of 0.5 mM-3-mercaptopicolinate (3-MPA) on NH₃ production from kidneys taken from rats perfused with 2 mM-aspartate and 5 mM-glucose

Control experiments are designated with broken lines and open symbols (*n* = 10). Where indicated, 0.5 mM-MPA was added to the perfusate (solid lines, filled symbols, *n* = 5). Rates of NH₃ production during the interval measured are shown at the top of the Figure. Key: ▽, ▴, NH₃; △, ▲, glutamate; ○, ●, glutamine; □, ■, aspartate.

ments do not demonstrate whether it is the purine nucleotide cycle or glutamate dehydrogenase that formed NH₃ from aspartate, neither do they demonstrate which pathway is inhibited by 3-MPA. To distinguish between the two pathways 3-MPA and a transaminase inhibitor (MVG) were tested in isolated kidney-tubule suspensions.

Experiments with tubule suspensions

Tubule suspensions, containing approx. 20 mg of protein, were prepared from acidotic rats and placed in 50 ml flasks containing O₂/CO₂ (19:1). At the start of a 60 min experiment glutamate or aspartate was added to the incubation medium to give a final concentration of 2 mM. In addition, either 5 mM-MVG or 1 mM-3-MPA, or both, were added to the incubation mixture and rates of production or uptake of NH₃, glucose, glutamate, aspartate, lactate and malate were determined. The results of these experiments are summarized in Table 2. The inhibitors, 3-MPA and MVG, had no effect on purine nucleotide cycle enzymes and glutamate dehydrogenase activities measured by previously published methods (Bogusky *et al.*, 1981).

Formation of NH₃ and glucose from 2 mM-glutamate was 1.54 ± 0.16 and 0.68 ± 0.05 nmol/min per mg of protein respectively. The addition of 3-MPA to the incubation flask inhibited formation of NH₃ and glucose and increased aspartate formation. In the presence of a transaminase inhibitor, NH₃ formation from glutamate is entirely via glutamate dehydrogenase. The addition of 3-MPA in experiments with MVG present inhibited glucose formation without inhibiting NH₃ formation. The presence of MVG completely prevented the increase in aspartate formation by 3-MPA. These data show that gluconeogenesis is not regulatory for NH₃ formation via glutamate dehydrogenase.

Table 2. Effect of 3-mercaptopicolinate and a transaminase inhibitor on metabolism of glutamate (Glu) or aspartate (Asp) in kidney tubule suspensions

Experimental details are given in the Methods section. Each value is the mean $\pm$ S.E.M. for six experiments. The rate of production or uptake (–) of metabolites is expressed in nmol/min per mg of protein. Significance values are for the effect of 3-MPA versus conditions lacking 3-MPA. Abbreviation: NS, not significant.

	Ammonia	Glucose	Glutamate	Aspartate	Lactate	Malate
Glu	1.54 ± 0.16	0.68 ± 0.05	–0.98 ± 0.11	0.12 ± 0.02	0.06 ± 0.10	0.02 ± 0.07
Glu + 3-MPA	0.77 ± 0.10	0.06 ± 0.05	–0.50 ± 0.08	0.48 ± 0.03	0.24 ± 0.10	0.12 ± 0.10
P	<0.005	<0.001	<0.005	<0.001	NS	NS
Glu + MVG	1.67 ± 0.15	0.42 ± 0.05	–0.56 ± 0.16	0.25 ± 0.02	–0.10 ± 0.30	–0.11 ± 0.17
Glu + MVG + 3-MPA	1.49 ± 0.15	0.02 ± 0.04	–1.10 ± 0.34	0.24 ± 0.02	–0.20 ± 0.23	0.09 ± 0.22
P	NS	<0.001	NS	NS	NS	NS
Asp	1.71 ± 0.12		0.61 ± 0.25	–2.60 ± 0.16	0.04 ± 1.20	0.60 ± 0.24
Asp + 3-MPA	0.90 ± 0.08		0.67 ± 0.11	–1.85 ± 0.32	–2.21 ± 1.90	0.70 ± 0.35
P	<0.01		NS	<0.05	NS	NS
Asp + MVG	1.23 ± 0.14		0.08 ± 0.14	–2.51 ± 0.73	0.11 ± 0.08	0.05 ± 0.13
Asp + MVG + 3-MPA	0.65 ± 0.07		–0.57 ± 0.51	–0.58 ± 0.79	–0.10 ± 0.13	–0.08 ± 0.10
P	<0.01		NS	NS	NS	NS

When aspartate is provided to tubule suspensions, NH_3 is formed at a rate of $1.71 \pm 0.12 \text{ nmol/min per mg of protein}$. In the presence of a transaminase inhibitor, glutamate formation from aspartate is completely prevented ($P < 0.02$); therefore NH_3 formation under these conditions is entirely via the purine nucleotide cycle. NH_3 formation via the cycle is large and approximately equal to the rate of NH_3 formation from glutamate via glutamate dehydrogenase. Addition of 3-MPA to experiments with aspartate containing MVG inhibits NH_3 formation and aspartate uptake. These data show that 3-MPA inhibits NH_3 formation via the purine nucleotide cycle. Glucose was not made in experiments with aspartate. This cannot be entirely explained, since aspartate is a substrate for gluconeogenesis in other tissue preparations of kidney (Rognstad & Katz, 1970; Bogusky *et al.*, 1982).

These data help to explain our observations in perfused kidney. We conclude that NH_3 formation from aspartate in perfused kidney is entirely via the purine nucleotide cycle. This pathway probably operates at a low rate during normal acid-base balance *in vivo*, but providing aspartate to kidney accelerates adenylosuccinate synthetase (EC 6.3.4.4) (Henderson *et al.*, 1975) for NH_3 formation via the cycle. The probable mechanism of 3-MPA inhibition of ammoniogenesis *in vivo* is to raise tissue oxaloacetate concentrations. This diverts glutamate nitrogen to form aspartate, thus preventing NH_3 formation via glutamate dehydrogenase. An increase in aspartate would normally accelerate NH_3 formation via the purine nucleotide cycle; however, the aspartate thus formed is prevented from deamination, as this pathway is inhibited by 3-MPA. Hence PEPCK is not regulatory for renal NH_3 formation, but can strongly influence amino acid metabolism in kidney by determining oxaloacetate concentrations. At low oxaloacetate concentrations, such as during chronic metabolic acidosis in the rat (Vinay *et al.*, 1980), glutamate deamination is via glutamate dehydrogenase. At high oxaloacetate concentrations, such as during acute and chronic acidosis in the dog (Gougoux *et al.*, 1982; Vinay *et al.*, 1980), or during the earliest phases of acidosis in the rat (Vinay *et al.*, 1980), aspartate may be formed from glutamate for deamination via the purine nucleotide cycle. This interpretation may also explain the metabolic effect of 3-aminopicolinate. This compound raises kidney tissue aspartate content and stimulates formation of NH_3 and glucose *in vivo* from glutamine in rats (Man *et al.*, 1981).

We show that gluconeogenesis is not regulatory for ammonia formation in kidney. We postulate that the role of PEPCK in kidney is to regulate tissue oxaloacetate concentrations. The concentration of oxaloacetate then determines whether glutamate is

deaminated via glutamate dehydrogenase or transaminated to aspartate for deamination via the purine nucleotide cycle. Except for the hydrolysis of GTP, NH_3 formation from glutamate via glutamate dehydrogenase and via the purine nucleotide cycle is virtually indistinguishable (Lowenstein, 1972). The purine nucleotide cycle can form NH_3 at the rate of $1.0 \mu\text{mol/min per g of kidney in rat}$ and may play a significant role in renal NH_3 production in this and other species.

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