The purine nucleotide cycle and ammonia formation from glutamine by rat kidney slices

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To test the significance of the purine nucleotide cycle in renal ammoniagenesis, studies were conducted with rat kidney cortical slices using glutamate or glutamine labelled in the α -amino group with ^{15}N . Glucose production by normal kidney slices with 2 mM-glutamine was equal to that with 3 mM-glutamate. With L-[^{15}N]glutamate as sole substrate, one-third of the total ammonia produced by kidney slices was labelled, indicating significant deamination of glutamate or other amino acids from the cellular pool. Ammonia produced from the amino group of L-[α - ^{15}N]glutamine was 4-fold higher than from glutamate at similar glucose production rates. Glucose and ammonia formation from glutamine by kidney slices obtained from rats with chronic metabolic acidosis was found to be 70% higher than by normal kidney slices. The contribution of the amino group of glutamine to total ammonia production was similar in both types of kidneys. No ^{15}N was found in the amino group of adenine nucleotides after incubation of kidney slices from normal or chronically acidotic rats with labelled glutamine. Addition of P_i , a strong inhibitor of AMP deaminase, had no effect on ammonia formation from glutamine. Likewise, fructose, which may induce a decrease in endogenous P_i , had no effect on ammonia formation. The data obtained suggest that the contribution of the purine nucleotide cycle to ammonia formation from glutamine in rat renal tissue is insignificant.

It is well documented that ammoniagenesis in the kidney occurs mainly by deamidation of glutamine in the mitochondria. However, it is still controversial whether ammonia formed from the amino group of glutamine is liberated only in the mitochondria by glutamate dehydrogenase or also in the cytosol by the purine nucleotide cycle (see review by Tannen, 1978), the other metabolic pathway for ammonia formation (Parnas *et al.*, 1927; Lowenstein, 1972).

Isolated rat renal mitochondria incubated with glutamate produce aspartate predominantly. However, mitochondria incubated with glutamine also produce aspartate in a significant amount (Kovačević, 1971; Strzelecki & Rogulski, 1976; Schoolwerth *et al.*, 1978). Aspartate formed from glutamine can subsequently be deaminated in renal cells. Thus, it is not possible to exclude a contribution of the purine nucleotide cycle activity to renal ammoniagenesis on the basis of lack of observed aspartate accumulation during metabolism of glutamine by isolated rat renal tubules (Baverel & Lund, 1979). The inhibition by amino-oxyacetate of nitrogen flux from the amino group of glutamine into the purine nucleotide cycle had no effect on ammonia formation by kidney cortical slices (Narins

& Relman, 1974). However, it was subsequently noted that glutamine metabolism by mitochondria upon amino-oxyacetate addition appears to be dependent on experimental conditions, e.g. glutamine present as sole substrate or with additional substrates (Schoolwerth & LaNoue, 1980; Strzelecki *et al.*, 1980).

The enzymes of the purine nucleotide cycle have much lower activities in kidney than in skeletal muscle, where this cycle is very efficient (Purzycka, 1962; Lowenstein, 1972; Muirhead & Bishop, 1974). However, the activity of adenylosuccinate synthetase, the rate-limiting enzyme in the purine nucleotide cycle, is increased in metabolic acidosis (Bogusky *et al.*, 1976).

In an attempt to elucidate the contribution of the purine nucleotide cycle to ammonia production from glutamine in kidney, the flux of ^{15}N from glutamine to the amino group of adenine nucleotides was measured in renal slices from normal and chronically acidotic rats.

Materials and methods

Experimental

Wistar rats (180–250 g) were given free access to

food and water. Chronic metabolic acidosis was induced by giving 1.5% NH_4Cl for 7 days in the drinking water.

The kidneys were removed and placed in a cold (4°C) solution of 140 mM-NaCl plus 5 mM-KCl and cortical slices were prepared manually with a razor over a period 15–20 min. The slices (50–80 mg wet weight) were incubated for 60 min at 37°C in Erlenmeyer flasks containing 4.0 ml of the following medium: 120 mM-NaCl, 23 mM- NaHCO_3 , 4.5 mM-KCl, 1.3 mM- CaCl_2 , 0.6 mM- MgSO_4 , 1.2 mM- NaH_2PO_4 at pH 7.4 and equilibrated with CO_2/O_2 (1:19, v/v). The substrates were introduced into the medium as indicated in the legends to the Tables. In experiments with labelled glutamate or glutamine, slices weighing 150 mg wet wt. were incubated in 7.0 ml of the medium. At various times, 0.5 ml portions of incubation medium were withdrawn and added to 0.1 ml of HClO_4 (24% w/v) for the assay of metabolites. To determine adenine nucleotide content in slices after incubation with labelled amino acids, slices were drained on a gauze layer placed on a funnel connected to a vacuum. The slices were transferred to 0.5 ml of HClO_4 (10%, w/v) in a glass microhomogenizer. The total time for separation of the slices was 7–20 s.

Chemicals

L-[^{15}N]Glutamate was synthesized by a modification of a method described by Rogulski & Angielski (1963) and Pitts *et al.* (1965). The reactions involved include:

The glutamate synthesis was carried out in a medium containing 60 mM-triethanolamine buffer, pH 8.0, 6 mM- MgCl_2 , 5 mM-2-mercaptoethanol, 35 mM-2-oxoglutarate, 50 mM- $^{15}\text{NH}_4\text{Cl}$, 0.6 mM- NADP^+ , 50 mM-glucose and 15 mM-ATP, in a total volume of 30 ml. 2-Oxoglutarate (POCH, Gliwice, Poland) was purified by the method of Krebs *et al.* (1961). $^{15}\text{NH}_4\text{Cl}$ containing 96.3% ^{15}N was obtained from VEB, Berlin, East Germany, and NADP^+ from Sigma Chemical Co. Enzymes added to this medium included glutamate dehydrogenase (Boehringer G.m.b.H.) in glycerol solution (10 units/ml), hexokinase (Sigma; 5 units/ml) and glucose 6-phosphate dehydrogenase (Sigma; dialysed in ammonia-free 20 mM-phosphate buffer, pH 6.7; approx. 20 units/ml). The content of $^{14}\text{NH}_4^+$ in the medium before $^{15}\text{NH}_4\text{Cl}$ addition was below 0.1 mM. The reaction

mixture was incubated for 3–3.5 h at 37°C and small portions of NaOH were added successively to maintain pH values in the range 7.8–8.1. The glutamate synthesized was desalted by column chromatography on a Dowex 50W (X8; Cl^- form) column (1.5 cm $\times$ 11 cm) by the method of Simon *et al.* (1967) and evaporated to dryness at 50°C . It was resuspended, neutralized with NaOH and assayed enzymically (Bernt & Bergmeyer, 1965). The yield from the complete procedure was 75%.

L-[α - ^{15}N]Glutamine synthesis was performed by the procedure of Brosnan & Hall (1977) in a medium containing 15 mM-imidazole buffer, pH 7.2, 20 mM- MgCl_2 , 10 mM-2-mercaptoethanol, 35 mM-ATP, 100 mM- NH_4Cl , 25 mM-L-[α - ^{15}N]glutamate (sodium salt) and partially purified glutamine synthetase from sheep brain (Rowe *et al.*, 1970) in a final volume of 25 ml. This mixture was incubated for 3.5 h at 37°C . At the end of incubation, the medium was applied to a combination of two columns: Amberlite XAD-2 (2.2 cm $\times$ 4.5 cm) placed over a column of Dowex 1 (X4; 1.6 cm $\times$ 13 cm; formate form) by the methods of Nieman *et al.* (1978) and Adam & Simpson (1974) respectively. Glutamine, essentially free of ATP and ADP, was eluted with water. The effluent was monitored by the reaction of one drop of eluate with ninhydrin reagent on Whatman no. 1 paper. Then, 0.5 vol. of chloroform/methanol (3:2, v/v) was added to the eluate, shaken vigorously and centrifuged for 3 min at 3500 g. The clear supernatant was evaporated at 50°C and the residue was dissolved in a small amount of water. A two-step

recrystallization of glutamine from ethanol was performed. Glutamine obtained in this way was tested for purity by t.l.c. on silica-gel plates in two solvent systems, methanol/pyridine/water (20:1:5, by vol.) and propan-2-ol/acetic acid/water (20:1:5, by vol.) and determined by an enzymic method (Meister, 1955). Glutamine was synthesized at a yield of 35%.

Determination of metabolites

Glucose was determined enzymically in the neutral HClO_4 extract of the incubation medium by the 'GOD-Perid Method-kit' from Boehringer. Ammonia was determined by the indophenol reaction (Okuda *et al.*, 1965) and by an enzymic ' NH_3 -kit' (Boehringer). The concentration of adenine nucleotides in the slices after incubation was assayed in

HClO₄ extracts, neutralized with solid KHCO₃ to pH 6.5 in the presence of Phenol Red. A portion (0.1 ml) of the neutralized extract was used for determination of ATP, ADP and AMP by enzymic methods (Adam, 1965; Lamprecht & Trautschold, 1965) on an Eppendorf colorimeter in micro-cuvettes with an optical pathlength of 2 cm. From the remainder of the neutralized extract, a 0.4 ml portion was applied to a Sephadex G-10 column (0.9 cm × 1.0 cm) and eluted with ammonia-free water. The nucleotide fraction (0.9–1.3 ml), monitored spectrophotometrically at 260 nm, was collected and used directly for degradation of adenine nucleotides to ammonia.

Degradation of ATP, ADP and AMP to ammonia was performed by the method of Munch-Petersen & Kalckar (1957) in a medium containing 70 mM-succinate buffer, pH 6.2, 6 mM-MgCl₂ and the following enzymes: potato apyrase isolated by the procedure of Krishnan (1956), myokinase (Sigma) dialysed in EDTA buffer (0.01 M, pH 6.4, free of ammonia) and adenylate deaminase isolated from chicken skeletal muscle as described by Stankiewicz *et al.* (1978). Ammonia in the reaction mixture (1.2–1.6 ml) was assayed at the beginning and at the end of incubation conducted overnight at room temperature. Ammonia liberated in the sample minus ammonia formed in the blank was used for calculation of the adenine nucleotide content in the slices and was compared with the sum of ATP, ADP and AMP obtained by enzymic methods to assess the efficiency of the degradation procedure.

$$\text{Ammonia from amino group} = \Delta\text{NH}_3 \times (\text{N-NH}_3)/(\text{N-Gln})$$

Determination of ¹⁵N in NH₄⁺

The medium after incubation of slices or after adenine nucleotide degradation was mixed with an equal volume of saturated solution of K₂CO₃ in small glass bottles. A standardized solution of NH₄Cl containing various known amounts of ¹⁵N was prepared for each experiment and run through the analytical procedure. The bottles were stoppered immediately and ammonia was trapped overnight in 25 μl of 1 M-HCl placed in a vial inside the bottle. The determination of ¹⁵N was performed on a Varian MAT-711 mass spectrometer. The temperature of the sample was 120°C, ionization energy was 70 mV, temperature of source 260°C and sensitivity 3500. The natural abundance of ¹⁵N was not

[NH₄⁺ appearing in the medium after deamination of adenine nucleotides (0.22 mM)] × [diluting factor (1.5/0.4)]/[the weight of slices in 1.0 ml of the neutralized extract (141 mg/0.5 ml)].

detectable under these conditions. The ¹⁵NH₄⁺ peak at *m/z* 18 was located at the end of the H₂O peak, if the sensitivity was set at 3500. The method of 'peak matching' was applied to estimate the contribution of *m/z* 17 (¹⁴NH₄⁺) and *m/z* 18 (¹⁵NH₄⁺) in the total NH₄⁺.

Results

It has been reported that P_i is a strong inhibitor of AMP deaminase, the key enzyme in the purine nucleotide cycle (Lowenstein, 1972). Fig. 1 shows that ammonia formation by kidney cortical slices with 2 mM-aspartate decreased markedly as medium phosphate was elevated from 0.3 to 6.0 mM. With glutamine, ammonia production by renal slices was independent of the change in phosphate concentration, consistent with previous findings (Goldstein & Schooler, 1967). Addition of fructose to the medium containing either glutamine or aspartate stimulated glucose production, whereas ammonia formation remained unchanged (Table 1). Fructose would be expected to lower endogenous phosphate (Van den Berghe *et al.*, 1977).

Tables 2 and 3 present results obtained with glutamine of glutamate labelled with ¹⁵N in the amino nitrogen atom. The data obtained in one experiment are presented in detail in Table 2 to show how the calculation was performed.

In the preliminary experiments, no ¹⁵N was detected in NH₄⁺ at the beginning and after incubation for 1 h of the incubation medium containing labelled glutamine without slices. At the end of incubation of slices with labelled glutamine, the contribution of ¹⁵N in NH₄⁺ (1.25 mM) was 16% and the content of ¹⁵N in the ammonia formed was 20%. Ammonia formation from the amino group of glutamine (or glutamate) was estimated on the basis of isotope flux as follows:

where ΔNH₃ denotes total ammonia formation by kidney slices, N-NH₃ represents the percentage of ¹⁵N in the ammonia formed and N-Gln represents the percentage of ¹⁵N in the amino group of glutamine or glutamate.

Ammonia liberated from AMP in the purine nucleotide cycle comes from the amino group at position 6 in the purine ring. Since the AMP formed from aspartate by condensation with IMP may easily enter the metabolic pool of adenine nucleotides in the tissue (via myokinase), the labelled nitrogen was determined in total adenine nucleotides. In order to assess the efficiency of the procedure the concentration of adenine nucleotide in slices was calculated from:

The value (2.9 μmol/g wet wt.), not shown in the Table, agrees well with the sum of ATP, ADP and AMP obtained by separate determination using the enzymic methods.

The contribution of ¹⁵N in NH₄⁺ was found to be less than 2% in the final degradation medium. Since

Fig. 1. Effect of P_i on ammonia formation from aspartate and from glutamine by rat kidney slices

Rat renal cortical slices were incubated in Krebs-Henseleit medium at pH 7.4 containing 2 mM-aspartate or glutamine and various amounts of Na_2HPO_4 . Values are means $\pm$ S.E.M. from four to eight experiments.

Table 1. Effect of D-fructose on glucose and ammonia formation by kidney slices

Rat renal cortical slices were incubated in Krebs-Henseleit medium at pH 7.4 with 2 mM-aspartate or -glutamine and with or without 5 mM-D-fructose. Results are expressed as means $\pm$ S.E.M. from four experiments.

Substrates	Production ($\mu\text{mol/g}$ wet wt. per 60 min)	
	Glucose	Ammonia
Aspartate	6.2 ± 1.6	32.3 ± 12.3
Aspartate + fructose	41.6 ± 12.1	32.0 ± 8.0
Glutamine	10.2 ± 1.1	47.4 ± 2.9
Glutamine + fructose	39.5 ± 2.8	42.0 ± 3.6

about half of the ammonia in the final degradation medium comes from adenine nucleotides, the ^{15}N in adenine nucleotides is expressed below 5% (Table 2).

The content of ^{15}N in the amino group of adenine nucleotides should be equal to the amount of isotope incorporated into the adenine nucleotide pool divided by pool size, as follows:

$$\text{Specific activity} = \frac{\text{amount of isotope}}{\text{amount of substrate}} = \frac{\Delta \text{Gln} \times (\text{N-Gln})}{\text{Ade}}$$

where ΔGln represents the influx of nitrogen from the amino group of glutamine, N-Gln the percentage of ^{15}N in its amino group and Ade the sum of ATP, ADP and AMP in slices after incubation.

The metabolic pool of adenine nucleotides in the slices was assumed to be constant, because insignificant changes were found in the tissue nucleotide content after incubation from one experiment to another. The average of the determined values of

adenine nucleotides in slices was $2.1 \mu\text{mol/g}$ wet wt. and glutamine consumption was $40 \mu\text{mol/g}$ wet wt. per 60 min. If this glutamine consumption is assigned an arbitrary value of 1.0 unit, then the adenine nucleotide pool is 0.05. Moreover, if the flux of nitrogen into the adenine nucleotide pool is set at a rate 0.01 of the glutamine consumed and the content of ^{15}N is set at 100%, 50% or 10% of total nitrogen in the amino group of glutamine, it should be possible to detect 20%, 10% or 2% of labelled nitrogen in the amino group of adenine nucleotides respectively. In the experiment presented in Table 2, the flux of nitrogen through the purine nucleotide cycle would be less than 1% of the glutamine consumed, below $0.4 \mu\text{mol/g}$ wet wt. per 60 min, the limit of detection.

Rat kidney cortical slices obtained from animals with normal acid-base balance produced 1.5 times more ammonia than glucose with 3 mM-glutamate as sole substrate (Table 3). The nitrogen from the amino group of glutamate contributed approx. one-third of the total ammonia production. This would suggest that ammonia can be produced in kidney not only by deamination of glutamate but also in part by deamination of other monoamino-monocarboxylic acids by L-amino acid oxidase (Davies & Yudkin, 1952). The fact that ammonia is produced from endogenous unlabelled amino acids is confirmed by the observation (Fig. 1) that ammonia formation in the absence of added glutamate (endogenous) is approx. two-thirds of the value (Table 3) obtained with added (3 mM) glutamate. Thus the additional ammonia produced by adding [^{15}N]glutamate to the media is almost completely labelled.

Glucose production by kidney cortical slices with 2 mM-glutamine was equal to that with 3 mM-glutamate. However, the rate of ammonia formation was three times higher. The amino group of glutamine appeared to be the source of about one-third of total ammonia produced. This suggests that in kidney slices one-half of the glutamate formed from glutamine is deaminated and serves as a source for glucose synthesis, whereas the remaining glutamate enters the cellular pool without

producing ammonia. The addition of D-fructose to the medium with glutamine had no effect on total ammonia formation, but ammonia production from the amino group of glutamine decreased markedly.

The production of ammonia and glucose by kidney slices obtained from rats with chronic metabolic acidosis was 70% higher than by normal rat kidney slices. The percentage contribution of the amino group of glutamine to total ammonia produc-

Table 2. The experimental data and calculated values of ¹⁵N flux from the amino group of glutamine in renal cortical slices of normal rat

The experimental conditions are reported in the Materials and methods section and the calculation procedure is described in the text.

Renal cortical slices, 141 mg wet wt.	
Krebs–Henseleit medium, pH 7.42; total volume of 7.0 ml	
2 mM-glutamine, 63% ¹⁵ N in the amino group	
Glucose production	10.9 μmol/g wet wt. per 60 min
Ammonia formation	
NH ₄ ⁺ in the incubation medium	initial
	final
	0.26 mM
	1.25 mM
Total ammonia production	49.7 μmol/g wet wt. per 60 min
Ammonia formed from the amino group	15.7 μmol/g wet wt. per 60 min
Ammonia formed via the purine nucleotide cycle	below 0.4 μmol/g wet wt. per 60 min
Adenine nucleotides in slices after incubation	
ATP	0.89 μmol/g wet wt.
ADP	1.55 μmol/g wet wt.
AMP	0.39 μmol/g wet wt.
NH ₄ ⁺ in the degradation medium (1.5 ml)	
NH ₄ ⁺ increase in the blank sample	0.08 mM
Initial concentration	0.18 mM
Final concentration	0.48 mM
Adenine nucleotide concentration (0.4 ml)	0.82 mM
Mass spectrogram of NH ₄ ⁺	
The incubation medium, final	
Peak of <i>m/z</i> 17	87 mm
Peak of <i>m/z</i> 18	16 mm
¹⁵ N in the ammonia formed	20%
The degradation medium, final	
Peak of <i>m/z</i> 17	61 mm
Peak of <i>m/z</i> 18	1 mm
¹⁵ N in adenine nucleotide	below 5%

Table 3. Ammonia formation from glutamate or glutamine by renal cortical slices of normal rats (serum HCO₃⁻ 26 m-equiv./litre) and of rats with chronic metabolic acidosis (serum HCO₃⁻ 18 m-equiv./litre) and the flux of nitrogen from the amino group of these amino acids into tissue adenine nucleotides

Rat renal slices were incubated in Krebs–Henseleit medium, pH 7.4, at 37°C. The values represent data obtained from separate experiments. The percentage of ¹⁵N in the amino group of amino acids is shown in parentheses.

	Production (μmol/g wet wt. per 60 min)			Glutamine utilization (μmol/g wet wt. per 60 min)	¹⁵ N (%) in:	
	Glucose	Ammonia total	¹⁵ N-NH ₃		Ammonia formed	Adenine nucleotides
Normal rats						
L-[¹⁵ N]Glutamate at 3 mM (91)	10.4	15.2	4.7	—	28	<5
	10.4	16.5	5.7	—	33	<5
L-[α- ¹⁵ N]Glutamine at 2 mM (71)	11.0	51.4	16.6	36.0	23	<5
	9.4	51.5	22.5	31.0	31	<5
L-[α- ¹⁵ N]Glutamine at 2 mM plus fructose at 5 mM	37.6	41.2	10.4	30.8	18	<5
Acidotic rats						
L-[α- ¹⁵ N]Glutamine at 2 mM (71)	17.6	84.5	29.7	49.5	25	<5
	18.5	87.5	29.5	47.5	24	<5

tion was similar in both types of kidneys. The rates of glucose and ammonia production by kidney slices from normal and chronically acidotic rats agree well with data reported by Preuss *et al.* (1973) under the

same experimental conditions. Similarly, calculation of ammonia formation from the amino group of glutamine based on the isotopic flux are in accordance with the values obtained from the nitrogen

balance. This is in contrast with the data obtained with isolated renal tubules, which revealed a greater capacity to deaminate glutamine (Vinay *et al.*, 1978; Baverel & Lund, 1979).

The metabolic route of nitrogen from the amino group of glutamine to ammonia through the purine nucleotide cycle was tested by determination of the amount of ^{15}N in position 6 of the purine ring of AMP, ADP and ATP in slices after incubation with labelled glutamine. Under the experimental conditions presented in Table 3 one would expect more than 20% of the amino group of adenine nucleotides to be labelled at flux rates above 2% of glutamine consumed. The results obtained are much below this value, indicating insignificant flux of nitrogen from the amino group of glutamine to ammonia via the nucleotide pool in normal, as well as acidotic, rat kidney slices.

Discussion

The purpose of the present study was to test the hypothesis that cytosolic formation of ammonia from the amino group of glutamine occurs via the purine nucleotide cycle. It has been found in experiments with skeletal muscle that P_i is a strong inhibitor of AMP deaminase (Lowenstein, 1972). In the liver, decreased intracellular levels of P_i after a fructose load resulted in an increase in AMP deamination (Van den Berghe *et al.*, 1977), as also in the kidney, where sequestration of P_i as fructose 1-phosphate has been found to occur primarily in the proximal tubules (Burch *et al.*, 1980).

In kidney cortical slices, which contain a relatively high proportion of proximal tubules, it was demonstrated that ammonia formation from aspartate was inhibited by phosphate. However, fructose addition was without effect (Fig. 1, Table 1). Ammonia formation from glutamine by kidney slices was unaltered by either phosphate or fructose. Thus the glutamine deamidation pathway coupled with aspartate formation and subsequent deamination in the purine nucleotide cycle may be neglected, in contrast with the pathway for ammonia formation from exogenous aspartate, which remains unresolved.

Glutamate and aspartate are less effective substrates for renal ammoniogenesis than glutamine (Davies & Yudkin, 1952; Pitts *et al.*, 1965; Kamm & Strope, 1972; Klahr *et al.*, 1972). The data obtained in this study support the suggestion that extramitochondrial glutamate seems to be unavailable for ammonia formation by mitochondrial glutamate dehydrogenase, in contrast with glutamate formed intramitochondrially from glutamine (Kovačević, 1971; Strzelecki & Rogulski, 1976; Schoolwerth & LaNoue, 1980).

It has been reported that ^{15}N from aspartate readily appears in the amino group of adenine

nucleotides in rabbit skeletal muscle (Newton & Perry, 1960). When the amino group of aspartate is incorporated into AMP, ammonia may be liberated by AMP aminohydrolase (AMP deaminase; EC 3.5.4.6) or in the pathway catalysed sequentially by 5'-nucleotidase (EC 3.1.3.5) and adenosine deaminase (EC 3.5.4.4).

Both metabolic pathways are present in the kidney, where activities of AMP deaminase and adenosine deaminase are equal (Purzycka, 1962) and the activity of 5'-nucleotidase seems to dominate over them (Weidemann *et al.*, 1969). Amination of IMP by aspartate serves as a supply of nitrogen to the adenine nucleotide pool. The activity of adenylosuccinate synthetase, the rate-limiting enzyme of the purine nucleotide cycle, was reported to be 1% of the phosphate-dependent glutaminase activity (Bogusky *et al.*, 1981). With chronic acid feeding to rats, the activity of this enzyme rose 2-fold and paralleled an increase in urinary ammonia excretion. This might indicate that the purine nucleotide cycle plays a significant role in the adaptation of renal ammoniogenesis to chronic acidosis. However, the magnitude of the enzyme adaptation reported in the above paper was found to be small in comparison with the rise in phosphate-dependent glutaminase activity and to the increase in urine ammonia excretion.

The data obtained in this study indicate negligible flux of ^{15}N from the amino group of glutamine to adenine nucleotides in kidney slices from normal and chronically acidotic rats. Therefore it is suggested that deamidation and deamination of glutamine occurs predominantly in the mitochondrial compartment of renal cortical cells.

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