Early Events in the Initiation of Ammonia Formation in Kidney*

Ronald T. Bogusky and Thomas T. Aoki

From the Joslin Research Laboratory, Howard Hughes Medical Institute at Harvard Medical School, Peter Bent Brigham Hospital, Boston, Massachusetts 02215 and the Department of Internal Medicine, University of California Davis at Sacramento, California 95817

Experiments were designed to examine the early events in the initiation of glutamate deamination in kidney. Perfused kidneys from methionine sulfoximine-treated rats formed ammonia from $[^{15}\text{N}]$glutamate via the purine nucleotide cycle. The turnover of the 6-amino group of adenine nucleotides to yield ammonia occurred at the rate of 0.30 pmol/g of kidney/min. This rate is 3-4 times larger than in liver and is in agreement with published rates of the purine nucleotide cycle in kidney. In addition, 0.1 mM fluorocitrate blocked ammonia formation using glutamate dehydrogenase. The turnover of the 6-amino group of adenine nucleotides increased during the first 5 min after adding fluorocitrate to form ammonia predominately from tissue glutamate and aspartate. This turnover correlates with a 3-fold increase in kidney tissue (18). 13-20 The ATP/ADP ratio fell the purine nucleotide cycle was inhibited and glutamate dehydrogenase was inhibited to form ammonia stoichiometrically with glutamate taken up from the perfusate. Ammonia formation via glutamate dehydrogenase occurred at a rate of 1.0 pmol/g of kidney/min. Fluorocitrate completely blocked ammonia formation from aspartate in perfusions. The perfused kidney formed ammonia from aspartate via the purine nucleotide cycle at a rate of 1.0 pmol/g of kidney/min. The results indicate a discrete role for aspartate in renal metabolism. Ammonia formation via the purine nucleotide cycle can occur at significant rates and equal to the rate of ammonia formation from glutamate via glutamate dehydrogenase.

Several factors have been postulated to regulate ammonia production in kidney (1). One such factor regulating augmented ammonia formation during acidosis may be kidney tissue glutamate levels. Metabolite analysis of whole kidney demonstrates a fall in tissue glutamate content early in metabolic acidosis (2). This observation led to the hypothesis that the fall in glutamate content during acidosis removes the inhibition of phosphate-dependent glutaminase thereby promoting glutamine uptake and deamination by mitochondria (3, 4). Metabolic flux data in isolated mitochondria suggest that glutamate deamination via glutamate dehydrogenase may be important in determining tissue glutamate levels and, therefore, regulatory for renal ammoniagenesis (5, 6). The regulation of glutamate dehydrogenase has commanded considerable attention. However, a detailed study of this enzyme leading to the hypothesis that the fall in glutamate content during acidosis removes the inhibition of phosphate-dependent glutaminase thereby promoting glutamine uptake and deamination by mitochondria (3, 4). Metabolic flux data in isolated mitochondria suggest that glutamate deamination via glutamate dehydrogenase may be important in determining tissue glutamate levels and, therefore, regulatory for renal ammoniagenesis (5, 6). The regulation of glutamate dehydrogenase has commanded considerable attention. However, a detailed study of this enzyme...
of fluorocitrate. Perfused kidneys were stored immersed in liquid nitrogen until time of analysis, usually not longer than 1 week. Pentobarbital anesthetized kidneys were obtained according to the methods of Williamson and Corkey (15). Purified enzymes specific for substances measured were used to analyze neutralized samples of kidney extract.

To measure $^{15}$N incorporation into the 6-amino group of adenine nucleotides kidneys were perfused with $[^{15}$N]glutamate (Kor-isotope, Cambridge, MA), instantly frozen at timed intervals, and extracted with perchloric acid. The acid extract of kidney was titrated to pH 7.0 with 6 N KOH, and a small aliquot was removed for metabolite analyses. The remainder was titrated to pH 10 and then frozen and lyophilized to dryness. The residue was taken up in 2 ml of ammonia-free water, and the pH was adjusted to 7.0 with ammonia-free HCl (BDH catalog no. 19066). Two units of potato apyrase and 0.7 unit of AMP deaminase were then added. The mixture was incubated for 3 h, by which time the deamination of adenine nucleotides was complete. For the isolation of ammonia the solution was transferred quantitatively to the outer ring of a Conway diffusion unit. The center well contained 1 ml of freshly prepared ammonia-free 1 N HCl and 50 $\mu$l of 6 N KOH was added to the outer ring. The Conway units were kept at room temperature for 3 h with occasional shaking. The HCl solution was then analyzed for total ammonia and $^{15}$N atoms $\times$ excess. Perfused ammonia was distilled in Conway diffusion units as described above and analyzed for total ammonia and $^{15}$N atoms $\times$ excess. The samples from experiments were diluted with 10 mg of nitrogen (Analar NH$_4$Cl, BDH, -0.0021 atom $\times$ N compared to reference). The sample plus carrier were oxidized to N$_2$ by treating with alkaline hypobromite in cauco. The N$_2$ was then measured for $^{15}$N enrichment. Isotope abundances were determined relative to a laboratory standard on a VG Micromass 602E double collector mass spectrometer.

Assay—Ammonia was measured fluorometrically by the reductive amination of a-ketoglutarate via glutamate dehydrogenase (16). Glutamate was measured via glutamate dehydrogenase as was glutaminate, following glutaminase hydrolysis to glutamate (17). Aspartate was measured spectrophotometrically using a coupled reaction of glutamate-oxalacetate transaminase and malate dehydrogenase linked to NAD reduction at 340 nm (18). All enzymes and reagents were obtained from Sigma.

Lactate was measured according to the method of Gutman and Wahlefeld (19), pyruvate by the method of Czok and Lamprecht (20), citrate by the method of Passonneau and Brown (21), and a-ketoglutarate by the method of Narins and Passonneau (22). Malate was determined by the method of Gutman and Wahlefeld (23) and ATP by the method of Lamprecht and Trautschold (24). ADP and AMP were measured according to Jaworek et al. (25). Inosine, hypoxanthine, and xanthine were measured according to the methods of Kalckar (26). IMP was measured according to the method of Schultz and Lowenstein (27).

**RESULTS**

Ammonia Formation from $[^{15}$N]Glutamate—Kidneys from methionine sulfoximine-treated rats form ammonia from 5 mM glucose, pH 7.4, at the rate of 0.10 ± 0.03 $\mu$mol/g of kidney/min ($n = 9$). This rate increased to 0.35 $\mu$mol/g of kidney/min when 2 mM glutamate was added to the perfusate. Kidneys took up glutamate at a rate of 1.25 $\mu$mol/g of kidney/min during a 50-min perfusion. Between 20 and 50 min of perfusion, control kidneys took up 56 $\mu$mol of glutamate while making 6.5 $\mu$mol of ammonia leaving approximately 50 $\mu$mol of glutamate nitrogen unaccounted for as free ammonia (Fig. 1, broken lines). These kidneys were analyzed for ATP, ADP, AMP, and IMP during a 50-min perfusion (Table I). With $[^{15}$N]glutamate as substrate $^{15}$N incorporation into the 6-amino group of adenine nucleotides exceeds the appearance of $^{15}$N into free ammonia. This result indicates the purine nucleotide cycle is the primary source of ammonia formation from glutamate in these kidneys.

Effect of 0.1 mM Fluorocitrate on Ammonia Formation from $[^{15}$N]Glutamate—Fluorocitrate, a competitive inhibitor of aconitate (28), was added to perfusions to stimulate glutamate deamination. Addition of 0.1 mM fluorocitrate to rat kidney perfusions increased ammonia formation from 0.35 to

**TABLE I**

| Minutes of perfusion | ATP (nmol/g kidney) | ADP (nmol/g kidney) | AMP (nmol/g kidney) | IMP (nmol/g kidney) |
|----------------------|---------------------|---------------------|---------------------|---------------------|
| 20                   | 1.60 ± 0.08         | 0.77 ± 0.05         | 0.34 ± 0.04         | 12.5 ± 3.9          |
| 30                   | 1.57 ± 0.40         | 0.63 ± 0.11         | 0.39 ± 0.09         | 11.1 ± 4.0          |
| 40                   | 1.54 ± 0.28         | 0.77 ± 0.10         | 0.51 ± 0.13         | 10.0 ± 2.0          |
| 50                   | 1.40 ± 0.04         | 0.80 ± 0.12         | 0.46 ± 0.13         | 10.0 ± 1.0          |

$^{15}$N atom % excess in 6-amino group of adenine nucleotides

| $^{15}$N atom % excess in free NH$_3$ |
|-------------------------------------|
| 3%                                  |
| 7%                                  |
| 10%                                 |
| 13%                                 |
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1.2 μmol/g of kidney/min (Fig. 1, solid lines). After adding fluorocitrate at 20 min of perfusion, 39 μmol of ammonia were added to the perfusate during the 30 min following addition. During the same interval these kidneys took up 26 μmol of glutamate at a rate of 0.83 μmol/g of kidney/min.

Experiments with fluorocitrate were repeated except that the perfusions were interrupted by quick-freezing the kidneys after 20 min of perfusion, just before adding fluorocitrate, and at timed intervals after adding fluorocitrate. Fig. 2 shows the tissue content of glutamine, glutamate, aspartate, and ammonia in kidneys treated with fluorocitrate. The data in this figure are in micromoles/kidney. This presentation permits direct comparison with Fig. 1 and allows calculation of nitrogen balance from tissue plus perfusate. Immediately after adding fluorocitrate to the perfusate the tissue levels of all three amino acids fell rapidly while tissue ammonia increased by 2 μmol. Deamination of glutamate and aspartate accounts for 8.6 μmol of ammonia plus 2.4 μmol from both glutamine nitrogens resulting in a net yield of 11 μmol of ammonia from tissue amino acids. Glutaminase contributed very little to ammonia formation in this experiment since glutamine was not detected in the perfusate and tissue levels changed only slightly. Fig. 3 shows the tissue content of citrate, lactate, pyruvate, and α-ketoglutarate in kidneys perfused as described in Fig. 2. Addition of fluorocitrate caused a prompt rise in tissue citrate. The tissue contents of α-ketoglutarate and pyruvate changed very little after adding fluorocitrate while lactate levels fell and then returned to control levels by 40 min of perfusion.

To examine the effect of fluorocitrate on purine nucleotides in perfusions, ATP, ADP, AMP, and IMP were also measured in these same kidneys (Table II). After adding fluorocitrate, ATP fell by 42% in the first 5 min and fell by another 16% during the same interval these kidneys took up 20 μmol of lactate, pyruvate, and α-ketoglutarate in kidneys perfused as described in Fig. 2. Addition of fluorocitrate caused a prompt rise in tissue citrate. The tissue contents of α-ketoglutarate and pyruvate changed very little after adding fluorocitrate while lactate levels fell and then returned to control levels by 40 min of perfusion.

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![Fig. 2. Effect of 0.1 mM fluorocitrate on metabolites of perfused kidney.](http://www.jbc.org/)

**Fig. 2. Effect of 0.1 mM fluorocitrate on metabolites of perfused kidney.** Perffusions were stopped by freezing kidneys just before and at timed intervals after adding fluorocitrate to the perfusate. Kidneys were analyzed for glutamate, aspartate, glutamine, and NH₃. Results are expressed in μmol/kidney ± S.E. (n = 6 for each data point.)

**Fig. 3. Effect of 0.1 mM fluorocitrate on metabolites of perfused kidney.** The same kidneys in Fig. 2 were analyzed for citrate, lactate, pyruvate, and α-ketoglutarate. Results are expressed in μmol/g of kidney ± S.E.

**Table II**

**Effect of 0.1 mM fluorocitrate on metabolites of kidney perfused with [15N]glutamate**

Kidneys treated with 0.1 mM fluorocitrate were analyzed for ATP, ADP, and AMP, and the results are expressed in μmol/g of kidney ± S.E.). Tissue content of IMP is in nmol/g of kidney (±S.E.). (n = 6 for each time point). Listed are the atoms % excess of 15N from [15N]glutamate in the 6-amino group of adenine nucleotides and perfusate NH₃ at the time points indicated.

|          | 20       | 22%     | 25       | 27%     | 30       | 40       | 50       |
|----------|----------|---------|----------|---------|----------|----------|----------|
| **μmol/g kidney** |          |         |          |         |          |          |          |
| ATP      | 1.61 ± 0.01 | 1.23 ± 0.08 | 0.93 ± 0.06 | 0.96 ± 0.06 | 0.99 ± 0.21 | 0.85 ± 0.15 | 0.79 ± 0.06 |
| ADP      | 0.77 ± 0.05 | 0.69 ± 0.03 | 0.62 ± 0.03 | 0.60 ± 0.09 | 0.75 ± 0.06 | 0.66 ± 0.09 | 0.60 ± 0.02 |
| AMP      | 0.34 ± 0.04 | 0.38 ± 0.04 | 0.37 ± 0.03 | 0.37 ± 0.02 | 0.45 ± 0.01 | 0.46 ± 0.05 | 0.45 ± 0.02 |
| **nmol/g kidney** |          |         |          |         |          |          |          |
| IMP      | 12.6 ± 3.9  | 35 ± 4.6  | 42.4 ± 8.9  | 27.4 ± 6.2  | 12.0 ± 2.7  | <2.0     | 7.8 ± 1.7  |
| % atom excess of 15N in 6-amino group of adenine nucleotides | 8% | 13% | 27% | 13% | 8% | 9% | 10% |
| % atom excess in free NH₃ | 3% | 11% | 20% | 25% | 35% | 48% | 67% |
fluorocitrate was added of kidney/min during the first 5 min after adding fluorocitrate. The total amount of ammonia produced into perfusate and tissue after adding fluorocitrate was 41 pmol. This was exactly balanced by the renal uptake of glutamate from the perfusate plus the deamination of amino acids and nucleotides in tissue. Hence, the primary source of ammonia in these experiments was glutamate.

To determine the role of the purine nucleotide cycle in fluorocitrate perfusions, $^{15}$N incorporation into the 6-amino group of adenine nucleotides was measured and is presented in Table II. Ammonia formation from $[^{15}$N]glutamate increased significantly following addition of fluorocitrate. Timed with the rise in tissue IMP was a more than 5-fold rise in $^{15}$N incorporation into the 6-amino group of adenine nucleotides. This was exactly timed with the rapid fall in tissue glutamate when ammonia production started again, glutamate and aspartate fell. Thus, the early rise, fall, and rise of ammonia production was matched by a reciprocal change in tissue glutamate and aspartate levels. The small changes in tissue glutamine levels indicate glutaminase had not contributed significantly to the ammonia produced into the perfusate. Tissue citrate, lactate, $\alpha$-ketoglutarate, and pyruvate were the same as those depicted in Fig. 3 (not shown).

To examine the role of the purine nucleotide cycle in kidneys perfused with 1.0 mM fluorocitrate, ATP, ADP, AMP, and IMP were also measured in the same kidneys as in Fig. 5.

**Fig. 4. Effect of 1.0 mM fluorocitrate on ammonia production.** This experiment is the same as described in Fig. 1 except fluorocitrate was added to the perfusate to yield a final concentration of 1.0 mM ($n = 5$).

**Fig. 5. Effect of 1.0 mM fluorocitrate on amino acids of perfused kidney.** Perfusions were stopped by freezing kidneys just before and at timed intervals after adding fluorocitrate to the perfusate. Kidneys were analyzed for glutamate, aspartate, and glutamine. Results are expressed in $\mu$mol/kidney ± S.E. ($n = 6$ for each data point.)
Thus, with the fall in ATP was an increase in IMP and was a 3-fold rise in tissue IMP. At 27% min, when ammonia production into the perfusate stopped, the tissue levels of glutamate and aspartate. When ammonia production returned AMP fell and IMP rose again, in the tissue level of IMP. When ammonia production started again, the tissue levels of glutamate, aspartate, and AMP fell as IMP rose. These observations show that early in the initiation of ammonia formation in kidney deamination of tissue amino acids is via the purine nucleotide cycle and tissue IMP levels reflect cycle activity to deaminate tissue glutamate and aspartate. After 30 min of perfusion ammonia formation was via glutamate dehydrogenase from perfusate glutamate as ammonia formation via the cycle was inhibited.

**Effect of 0.1 mM fluorocitrate on ammonia production from aspartate**—Further proof of a role for the purine nucleotide cycle in renal metabolism was provided by experiments using aspartate as substrate. When kidneys were provided aspartate, ammonia was formed at a rate of 1.0 pmol/g of kidney/min. Ammonia production from aspartate fell after the addition of fluorocitrate (Fig. 6). The fall in ammonia production was matched by a decrease in renal uptake of aspartate without any change in perfusate glutamine or glutamate uptake. That neither glutamine nor glutamate were the source of ammonia in these perfusions is supported by the observation that perfusate levels of these two amino acids were the same despite major changes in ammonia production rates. Ammonia formation could not have occurred via glutamate dehydrogenase as we show this enzyme was stimulated by fluorocitrate to make ammonia. Thus, all the ammonia made from aspartate in these experiments arose from the purine nucleotide cycle.

**DISCUSSION**

The results of these experiments show that kidneys from normal rats treated with methionine sulfoximine form ammonia from glutamate via the purine nucleotide cycle at a rate of 0.3 μmol/g of kidney/min. Experiments with [15N] glutamate prove that the turnover of the 6-amino group of adenine nucleotides in rat kidney is substantial, approximately 3-4 times greater than in liver (29) and can account for the ammonia formed from glutamate in control perfusion experiments. This rate of ammonia formation from glutamate agrees with previously published enzyme rates of the purine nucleotide cycle during normal acid-base balance (30). Also in normal kidneys the addition of fluorocitrate to the perfusate acutely increased ammonia formation from glutamate to 1.2 μmol/g of kidney/min During the first 5-min interval after addition of fluorocitrate, the perfused kidney content of IMP increased 3-fold. This rise was associated with an increase in 15N enrichment into the 6-amino group of adenine nucleotide. The source of ammonia in these perfusions during the first 5 min after adding fluorocitrate appears to be tissue glutamate. Ammonia formation during this interval is probably via the purine nucleotide cycle. When the ATP/ADP ratio fell and, presumably, the GTP/GDP ratio, the turnover of the 6-amino group of adenine nucleotides was inhibited and ammonia formation then occurred via glutamate dehydrogenase from glutamate present in the perfusate. This result is consistent with the known ADP effect to activate glutamate dehydro-

**Table III**

| Minutes of perfusion | 20 | 22% | 25 | 27% | 30 | 40 | 50 |
|----------------------|----|-----|----|-----|----|----|----|
| **μmol/g kidney**    |    |     |    |     |    |    |    |
| ATP                  | 1.61 ± 0.02 | 1.11 ± 0.07 | 0.83 ± 0.06 | 0.79 ± 0.07 | 0.75 ± 0.04 | 0.75 ± 0.01 | 0.75 ± 0.01 |
| ADP                  | 0.77 ± 0.05 | 0.72 ± 0.07 | 0.73 ± 0.05 | 0.78 ± 0.01 | 0.70 ± 0.06 | 0.75 ± 0.01 | 0.75 ± 0.01 |
| AMP                  | 0.34 ± 0.04 | 0.33 ± 0.04 | 0.38 ± 0.04 | 0.46 ± 0.03 | 0.42 ± 0.06 | 0.28 ± 0.04 | 0.28 ± 0.04 |
| IMP                  | 12.5 ± 3.0 | 28.2 ± 7.1 | 37.2 ± 9.3 | 14.3 ± 8.9 | 21.4 ± 3.8 | 6 ± 1 |

**Fig. 6. Effect of 0.1 mM fluorocitrate on ammonia formation from aspartate.** Ammonia formation from kidneys taken from rats in normal acid-base balance perfused with 2 mM aspartate and 5 mM glucose (broken lines, hollow symbols, n = 10). Where indicated, 0.1 mM fluorocitrate was added to the perfusate (solid lines, closed symbols, n = 5).

and presented in Table III. The ATP level fell acutely during the first 5 min after fluorocitrate. Tided with the fall in ATP was a 3-fold rise in tissue IMP. At 27% min, when ammonia production stopped, the tissue level of IMP fell to the level as at 20 min of perfusion at the same time AMP rose 35%. When ammonia production returned AMP fell and IMP rose again. Thus, timed with the fall in ATP was an increase in IMP and a fall in tissue glutamate and aspartate. When ammonia production into the perfusate stopped, the tissue levels of glutamate, aspartate, and AMP rise simultaneous with a fall in the tissue level of IMP. When ammonia production started again, the tissue levels of glutamate, aspartate, and AMP fell...
A rise in IMP levels then stimulates glutamate deamination via the purine nucleotide cycle. In kidney, ADP formed as a result of ATP hydrolysis may also stimulate glutamate flux through glutamate dehydrogenase (31) and α-ketoglutarate dehydrogenase (36) for ammonia formation via this pathway. A physiologic circumstance in which cellular energy metabolism may be important in kidney is during the process of urinary acidification. Micropuncture techniques confirm that the mechanism of bicarbonate reabsorption and the accompanying production of an acid urine reside in the net secretion of hydrogen ions against a hydrogen ion gradient, the driving force being ATP and Na⁺ gradient dependent (37, 38).

Our observations with fluorocitrate differ from in vivo acidosis in two major respects. First, kidney tissue ATP levels remain unchanged from control during acute or chronic in vivo acidosis (39). However, stable ATP levels do not exclude the possibility that ATP hydrolysis or turnover may be increased in vivo. Tissue α-ketoglutarate levels are depressed and amino acid carbon is more rapidly disposed of, ultimately, as glucose (46, 41). These changes suggest an increase in flux via tricarboxylic acid cycle enzymes between α-ketoglutarate and oxaloacetate. The oxaloacetate thus formed is substrate for new glucose synthesis via phosphoenolpyruvate carboxykinase in kidney. Citrate oxidation is also increased in acidosis compared to controls (42, 43). This increase in tricarboxylic acid cycle flux may be to meet cellular energy needs. A second difference in our present observations is tissue IMP levels.

When aspartate was used as substrate in perfusion experiments, ammonia was formed at a rate of 1.0 pmol/g of kidney/min. This result confirms work by Pitts (33) that aspartate is a substrate that can form large quantities of ammonia in intact kidney. The addition of fluorocitrate to perfusates containing aspartate inhibited ammonia formation. Since fluorocitrate inhibited ammonia formation via the cycle while glutamate dehydrogenase was stimulated we conclude that ammonia formation from aspartate in perfused kidney occurred entirely via the purine nucleotide cycle. This result confirms that the purine nucleotide cycle can make ammonia from aspartate at the rate of 1.0 μmol/g of kidney/min which is equal to the rate of ammonia formation via glutamate dehydrogenase. While fluorocitrate only briefly increased ammonia formation from glutamate via the purine nucleotide cycle our observations prove that this pathway can be stimulated to form ammonia at substantial rates and can potentially form large quantities of ammonia at sustained rates if provided a continuous supply of aspartate in kidney at high ATP levels. These observations provide support for a potential discrete role for aspartate in renal ammoniagenesis.

These experiments also provide observations on the regulation of amino acid metabolism in mitochondria. We show that kidneys given fluorocitrate form ammonia via glutamate dehydrogenase stoichiometric with the amount of glutamate taken up from the perfusate. The stoichiometry proves that glutamate can directly penetrate to the inner mitochondrial matrix in the intact kidney cell and be utilized by glutamate dehydrogenase. This conclusion is contrary to observations using isolated mitochondria (7-9) that suggest only glutamate formed from the deamidation of glutamine is deaminated by glutamate dehydrogenase. In addition, our studies show that the fall in kidney tissue glutamate levels correlates exactly with an increase in 15N incorporation into the 6-amino group of adenine nucleotides. This correlation is evidence that the fall in tissue glutamate levels occurs primarily in the cytosolic compartment and, therefore, could not regulate mitochondrial glutaminase.

In previous work using fluorocitrate (34) it was postulated that glutamate dehydrogenase formed ammonia when tissue α-ketoglutarate levels fell after inhibition of citrate oxidation. Our data in rat kidney do not demonstrate a significant fall in tissue α-ketoglutarate levels. While α-ketoglutarate levels fall in the interval between 20 and 25 min of perfusion the change is small and the tissue levels returned to control values by 30 min when ammonia formation remains elevated. Thus, a fall in tissue α-ketoglutarate levels alone could not be the initiating event in ammonia formation via glutamate dehydrogenase. However, the rate of flow of metabolites through this pathway may be important as the results suggest a coordination of utilization and production to maintain α-ketoglutarate levels constant.

Our observations suggest a potential role for cellular energy metabolism in the regulation of amino acid metabolism in kidney. The stimulus to renal ammonia formation in these experiments appears to be the fall in ATP levels. It has been previously shown that exercise in the rat hindquarter is associated with a fall in the muscle content of ATP with a rise in IMP and ammonia production (35). The hydrolysis of ATP causes the myokinase reaction facilitated by AMP deaminase to preserve ATP levels. Thus

\[ 2 \text{ADP} \rightarrow \text{ATP} + \text{AMP} \rightarrow \text{IMP} + \text{NH}_3. \]

A rise in IMP levels then stimulates glutamate deamination via the purine nucleotide cycle. In kidney, ADP formed as a result of ATP hydrolysis may also stimulate glutamate flux through glutamate dehydrogenase (31) and α-ketoglutarate dehydrogenase (36) for ammonia formation via this pathway. A physiologic circumstance in which cellular energy metabolism may be important in kidney is during the process of urinary acidification. Micropuncture techniques confirm that the mechanism of bicarbonate reabsorption and the accompanying production of an acid urine reside in the net secretion of hydrogen ions against a hydrogen ion gradient, the driving force being ATP and Na⁺ gradient dependent (37, 38).

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The steady state levels of IMP in kidney are 5- to 9-fold increased in acidosis compared to controls (30). A rise in kidney tissue IMP levels, we demonstrate, is the result of increased ATP hydrolysis which is similar to observations in muscle (35) and brain (37). Elevated IMP levels in vivo would represent sustained purine nucleotide cycle activity in kidney to form ammonia.

In summary, we show that the purine nucleotide cycle can account for all the ammonia made from glutamate in isolated perfused kidney taken from rats in normal acid-base balance that have been pretreated with methionine sulfoximine. The purine nucleotide cycle and glutamate dehydrogenase can form ammonia at significant and approximately equal rates. The former pathway is inhibited by low ATP/ADP ratios while the latter is accelerated. The cycle operates to form ammonia until the ATP (and GTP) levels fall to some critical level at which point ammonia is formed via glutamate dehydrogenase. Perfusion glutamate can be deaminated via mitochondrial glutamate dehydrogenase directly without its transport into mitochondria as glutamine. Tissue glutamate largely reflects levels present in the cytosol and not mitochondrial compartment. Furthermore, we postulate that ATP hydrolysis stimulates ammonia formation in our experimental conditions. While the purine nucleotide cycle can form large quantities of ammonia in perfused kidney its role in the intact in vivo kidney has yet to be determined. It is possible that both the purine nucleotide cycle and glutamate dehydrogenase may contribute to ammonia formation from glutamate during in vivo acidosis since the two activities are virtually indistinguishable in tissue (44).

Acknowledgments—We wish to thank Dr. George F. Cahill, Jr., Dr. John M. Lowenstein, and Dr. Leah M. Lowenstein for their support and many helpful discussions. We wish to gratefully acknowledge the excellent technical assistance of Adacie Allen. We wish to thank Dr. Donald Phillips of the Department of Agronomy and Range Science, University of California, Davis, for performing the mass spectrometer analyses of nitrogen. We also gratefully acknowledge the expert secretarial help of Angelina Dorsey in the preparation of this manuscript.

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J. Biol. Chem. 1983, 258:2795-2801.

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