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The Effects of pH Change on Renal Ammoniagenesis *in Vitro*[1] (38195)

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Renal ammoniagenesis *in vitro* at medium pH 7.0 and 7.8 is of similar magnitude with glutamine as substrate (1, 2), whereas ammoniagenesis from glutamate is greater at the lower pH (3). Our purpose was to determine why such differences exist. To do this, we followed certain aspects of glutamine and glutamate metabolism by rat kidney slices at medium pH 7.0 and 7.8 under various conditions. We also followed the effects of pH on phosphate dependent glutaminase (PDG) activity in homogenates. We conclude that metabolism of glutamine by rat kidney slices at pH 7.0 and pH 7.8 is different despite a similar magnitude of ammonia production.

Materials and Methods. We used male Spague-Dawley rats weighing 250–300 g. These rats were fed Purina Rat Chow and H_2O *ad lib.* In some studies, the rats were given 2 intraperitoneal injections of methionine sulfoximine (20 mg/100 g) 17 and 4 hr prior to study. Two slices from each rat's kidney were cut on a Stadie–Riggs microtome and then bisected—each half approximated 30–40 mg. The medium was bicarbonate buffered so that the initial pH and pH following incubation in all studies approximated 7.0 and 7.8 (12 and 48 m*M* bicarbonate, respectively). One slice half was incubated at pH 7.0 and the other half at 7.8 for 90 min. Some media contained no substrate glutamine 2 m*M*, NH_4Cl 1 m*M*, glutamate 1 m*M* or 10 m*M*, and/or α-ketoglutarate 1 m*M*; and the gas phase was either 95% O_2–5% CO_2 or 95% N_2–5% CO_2. Glutamate was measured by the method of Meiss, Peyser and Miller (4). Phosphate dependent glutaminase I (PDG) activity was estimated using a procedure detailed by Janicki (5). The method of deproteinization of medium and estimations of ammonia and glucose have been described previously (6–8). pH was determined anaerobically using the expanded scale of a Copenhagen Radiometer pH meter, model 27. Results from both slice pairs were averaged to give one result, and the data compared statistically by paired analysis using Student's *t* test. Statistical significance was set at $P < .05$.

Results. Effects of pH on aerobic glutamine and glutamate metabolism. Slice studies performed in the bicarbonate-buffered media gassed with 95% O_2 and 5% CO_2 are shown in Table I. Without substrate, ammonia production from endogenous sources was not different at the pHs studied although gluconeogenesis was greater at the lower pH ($P < .01$). In agreement with others (1, 2), rat kidney slices incubated aerobically in the presence of glutamine showed no significant difference in renal ammoniagenesis but did show a significant increase in gluconeogenesis at the lower pH. Although ammoniagenesis with glutamine present was similar at either pH, glutamate accumulation was greater at pH 7.8. In contrast to glutamine and in agreement with previous findings (3), total ammonia production from glutamate was increased at pH 7.0 in the presence of

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TABLE I. Aerobic Ammoniagenesis and Gluconeogenesis by Rat Kidney Slices at Media pH 7.0 and 7.8.

			Ammonia production[a]				Glucose production[a]				Glutamate accumulation[a]			
Substrate	mM	# Rats	7.0	7.8	[c]	P[b]	7.0	7.8	[c]	P[b]	7.0	7.8	[c]	P[b]
None	0	8	11.3	11.2	+0.1 ± 0.5	NS	7.9	6.2	+1.7 ± 0.3	$<.01$	—	—	—	—
Glutamine	2	8	44.2	43.6	+0.6 ± 1.0	NS	9.7	6.3	+3.4 ± 0.1	$<.01$	9.5	15.3	−5.8 ± 0.8	$<.01$
Glutamate	10	6	27.2	18.5	+8.7 ± 0.9	$<.01$	14.6	10.4	+4.2 ± 0.3	$<.01$	—	—	—	—

[a] μmoles/g wet weight/90 min.
[b] NS—not significant $P > .05$.
[c] Average difference of pairs ± SEM.

TABLE II. Anaerobic Ammoniagenesis and Gluconeogenesis by Rat Kidney Slices at Media pH 7.0 and 7.8.

			Ammonia production[a]				Glucose production[a]				Glutamate accumulation[a]			
Substrate	mM	# Rats	7.0	7.8	[c]	P[b]	7.0	7.8	[c]	P[b]	7.0	7.8	[c]	P[b]
None	0	5	4.5	5.0	−0.5 ± 0.4	NS	0.5	0.5	0 ± 0.1	NS	—	—	—	—
Glutamine	2	6	27.8	44.9	−17.1 ± 0.9	$<.01$	1.2	1.0	+0.2 ± 0.2	NS	26.6	34.9	−8.3 ± 0.8	$<.01$
Glutamate	10	6	8.6	10.4	−1.8 ± 0.5	$<.02$	1.5	1.4	+0.1 ± 0.1	NS	—	—	—	—

[a] μmoles/g wet weight/90 min.
[b] NS—not significant $P > .05$.
[c] Average difference of pairs ± SEM.

TABLE III. Aerobic and Anaerobic Formation of Glutamine and Glutamate.

Substrate	mM	# Rats	Aerobic 7.0	Aerobic 7.8	Aerobic [c]	Aerobic P^b	Anaerobic 7.0	Anaerobic 7.8	Anaerobic [c]	Anaerobic P^b
			Glutamine appearance[a]							
NH_4^+ and Glutamate	1 and 1	4	6.1	5.2	$+0.9 \pm 8.0$	NS	4.4	3.9	$+0.5 \pm 5.7$	NS
			Glutamate appearance[a]							
NH_4^+ and α-Ketoglutarate	1 and 1	4	3.5	3.7	-0.2 ± 0.2	NS	13.2	13.4	-0.2 ± 0.2	NS

[a] μmoles/g wet weight/90 min.
[b] NS—not significant $P > .05$.
[c] Average difference of pairs ± SEM.

TABLE IV. Aerobic and Anaerobic Ammoniagenesis at Media pH 7.0 and 7.8 by Kidney Slices from Rats Receiving Methionine Sulfoximine.

Substrate	mM	# Rats	Ammonia production[a] 7.0	Ammonia production[a] 7.8	[b]	P	Glutamate accumulation[a] 7.0	Glutamate accumulation[a] 7.8	[b]	P
			Aerobic							
Glutamine	2	8	52.3	45.9	$+6.2 \pm 1.5$	$<.01$	6.1	14.8	-8.8 ± 0.6	$<.01$
			Anaerobic							
Glutamine	2	8	28.7	42.2	-17.4 ± 2.4	$<.01$	29.5	46.0	-16.5 ± 1.7	$<.01$

[a] μm/g wet wgt/90 min.
[b] Average difference of slice pairs ± SEM.

oxygen, and increased gluconeogenesis from glutamate at the lower pH also was apparent.

Effects of pH on anaerobic metabolism of glutamine and glutamate. While average aerobic ammonia production from glutamine was not statistically higher at the relatively more alkaline pH ($HCO_3^- = 48$ m*M*), a difference became obvious when the gas phase was changed to 95% N_2 and 5% CO_2 (Table II). Anaerobic ammoniagenesis in the presence of glutamine was 79% greater at pH 7.8 than 7.0. Glutamate accumulation rose at both pHs in N_2 but remained significantly higher at pH 7.8 than at 7.0. Anaerobic ammoniagenesis from exogenous glutamate was higher at pH 7.8.

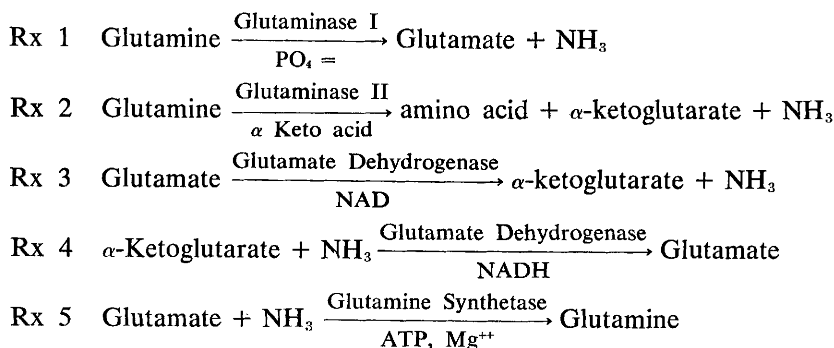
Aerobic and anaerobic formation of glutamate and glutamine at different pHs. When rat kidney slices were placed in medium containing 1 m*M* concentrations of NH_4Cl and α-ketoglutarate, the aerobic and anaerobic formation of glutamate were not different at the 2 pHs (Table III). In contrast, the production of glutamate was obviously enhanced in the N_2 compared to the O_2 environment. Starting with a different combination of substrates (1 m*M* glutamate and 1 m*M* NH_4Cl), glutamine appearance was not significantly different at the 2 pHs. Although the appearance of glutamine was lower in the N_2 compared to O_2, the differences did not prove to be statistically significant.

Effects of pH on aerobic and anaerobic glutamine and glutamate metabolism in slices from rats receiving methionine sulfoximine. Ammonia formation and glutamate accumulation from glutamine (2 m*M*) by kidney slices removed from rats receiving methionine sulfoximine were followed (Table IV). In this study a small but significant increase in aerobic ammonia formation was seen at pH 7.0 as compared to 7.8. However, glutamate accumulation aerobically and anaerobically and ammonia production anaerobically at the 2 pHs were similar to that seen in the tissues from control rats (Tables I and II).

Effects of pH on phosphate dependent glutaminase (PDG) activity. To study the effect of pH on PDG activity in whole kidney homogenates 7 samples were studied. PDG activity at pH 7.0 was .84 μmoles/g wet wt/hr $\pm$ .16 (SEM) compared to 2.0 μmoles/g wet wt/hr $\pm$ .22 (SEM) at pH 7.9.

Discussion. The data in Table I confirm earlier *in vitro* observations at pH 7.0 and 7.8—ammoniagenesis from endogenous sources or in the presence of glutamine differs little, but slice ammoniagenesis from exogenous glutamate is statistically greater at the lower pH (1–3). Despite a similar magnitude of ammoniagenesis from glutamine, glutamate accumulation is greater at pH 7.8. This coupled with greater gluconeogenesis at the lower pH suggests a difference in the metabolism of glutamine at the 2 pHs.

When studying renal glutamine metabolism by the rat, 5 reactions must be considered.



Because we have found that over 90% of the glutamine catabolized by rat renal slices forms glutamate at some time

$$\frac{\text{glutamate accumulation} + \text{glutamate deamination}}{\text{glutamine disappearance}}$$

(8), we agree with Goldstein (9) that Rx 1 rather than Rx 2 plays the major role in releasing ammonia from the amide nitrogen of glutamine. The appearance in N_2 of glutamate is nearly equal to ammonia formation (Tables II and IV) and supports further the above postulate. Eliminating Rx 2 from consideration, four possibilities could explain greater glutamate accumulation aerobically at pH 7.8 relative to pH 7.0: (1) an increase in glutamine deamidation (Rx 1); (2) a slower removal of the glutamate formed following deamidation (Rx 3); (3) an increased formation of glutamate through Rx 4; and/or (4) a slower removal of glutamate via decreased glutamine synthesis (Rx 5). Each possibility will be considered below.

(*1*) *An increase in glutamine deamidation.* Phosphate dependent glutaminase I (PDG) is a mitochondrial enzyme which has a pH optimum *in vitro* closer to 7.8 than 7.0 (10, 11). Our own studies show that PDG activity at pH 7.8 increases 138% over that seen at pH 7.0. Since PDG can deamidate glutamine in the absence of oxygen, ammonia derived from glutamine in a N_2 atmosphere reflects, to a great extent, the activity of the PDG pathway. By subtracting endogenous ammonia production from that produced in the presence of glutamine (Table II), substrate ammonia production can be estimated. Ammonia production from glutamine at pH 7.0 is 23.3 μm/g/90 min and at pH 7.8 is 39.9 μm/g/90 min. This is a 79% increase at the higher pH. Consistent with enhanced PDG activity at pH 7.8 is the larger and almost equal (to ammonia production) accumulation of glutamate at the higher pH in the nitrogen atmosphere. All this suggests that greater accumulation of glutamate at pH 7.8 could be secondary to greater deamidation (Rx 1) at this pH.

(*2*) *A slower removal of glutamate following deamidation.* In contrast to PDG, glutamate dehydrogenase (Rx 3) requires O_2 to continue oxidizing NADH to NAD. Inability to resupply this necessary coenzyme probably accounts for the relatively small deamination of exogenous glutamate in N_2 (Table II). To counterbalance increased ammonia production via deamidation at pH 7.8, increased oxidative deamination at pH 7.0 of glutamate formed following glutamine deamidation could account for the equimolar production of ammonia from glutamine at the 2 pHs. Greater gluconeogenesis at the lower pH (Tables I and IV) suggests that this is true. Finally, the greater ammoniagenesis from exogenous glutamate at pH 7.0 (Table I) is also consistent with enhancement of Rx 3. We conclude that slower deamination of glutamate at pH 7.8 could contribute to greater accumulation of this end product.

(*3*) *An increased formation of glutamate from α-ketoglutarate and ammonia.* We also checked to see whether Rx 4, i.e., greater net glutamate formation from α-ketoglutarate and NH_4^+ at pH 7.8 could account for increased glutamate. As shown in Table III, this reaction is not affected by slices incubated at the 2 pHs. In contrast, the accumulation of glutamate was greater anaerobically and was due most likely to increased concentrations of NADH and/or NADPH. If so, this indicates the importance of the pyridine nucleotide system in controlling the glutamate dehydrogenase pathway in rat kidney slices (Rx 3 and 4). We conclude that this reaction does not contribute greatly to increased glutamate accumulation at pH 7.8.

(*4*) *An increased formation of glutamine from glutamate and ammonia.* We considered that increased utilization of glutamate to synthesize glutamine (Rx 5) at pH 7.0 could lower its concentration. When both glutamate and NH_4^+ were added to incubating slices, we could see no difference in the

appearance of glutamine at the two pHs, aerobically or anaerobically. Further, slices from rats receiving methionine sulfoximine, an agent which decreases renal glutamine synthetase (Rx 5) and glutamine transferase activity approximately 2/3 (12), did not drastically change glutamate accumulation results. (Compare Tables I, II, and IV.) One difference in this study compared to the earlier one (Table I) was that ammoniagenesis from glutamine at pH 7.0 was greater than at 7.8. We do not feel that this difference is secondary to a decrease in glutamine synthetase activity. If decreased synthetase activity (Rx 5) in methionine sulfoximine injected rats caused this difference, not only ammonia formation would increase but glutamate accumulation should increase at pH 7.0 (Rx 5). (Compare Tables I and IV.) Rather, the accumulation of glutamate is even less than in the original studies depicted in Table I. We conclude that changes in the glutamine synthetase pathway are not responsible for greater glutamate accumulation at pH 7.8.

Rat kidney slices produce ammonia from both the amide and amino nitrogens (8). From these data, it is evident that the processes which contribute to almost equal ammonia production by rat kidney slices at media pH 7.0 and 7.8 are not quantitatively the same. As a first approximation, it seems reasonable to say that amide ammonia formation is greater at pH 7.8 and amino ammonia formation at pH 7.0. These studies suggest that lowering systemic pH and/or HCO_3^- concentrations alone can account, at least in part, for lower glutamate concentrations seen in acidotic rat kidneys, but cannot account for the lower glutamine concentrations also seen (9).

Summary. Despite similar ammonia production at pH 7.0 and 7.8 by rat kidney slices, greater glutamate accumulation at pH 7.8 suggests that renal glutamine metabolism differs at these 2 pHs. Based on a number of observations, we conclude that there is greater glutamine deamidation at pH 7.8 which is balanced by a greater deamination of formed glutamate at pH 7.0. Since glutamine and glutamate concentrations are decreased in the kidneys of acidotic rats, other factors besides pH and HCO_3^- concentrations must modify deamidation, whereas, pH and HCO_3^- concentrations may have a decided effect in lowering glutamate concentrations via enhanced deamination.

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