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Renal Phosphate-Dependent Glutaminase Activity and Ammonia Excretion during Acute Acidosis in the Rat (40529)

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The renal adaptation to an acid load in the rat has been extensively investigated. The two primary features of this adaptation are (i) a 100-fold increase in ammonia production and excretion, and (ii) a severalfold rise in phosphate-dependent glutaminase (PDG) activity. Although it had been suspected previously (1), Davies and Yudkin (2) first demonstrated both an increase in ammonia excretion and in PDG activity following chronic acid loading in rats. Rector *et al.* (3) then demonstrated a parallel rise in ammonia excretion and PDG activity for several days following the first 24 hr after NH_4Cl administration in rats. Results of these studies led to the conclusion that the rise in PDG activity during several days of acid challenge was partly responsible for the increase in ammonia excretion during the same period. Contrary to this, Goldstein (4) demonstrated that actinomycin D administration to adult rats prevented the adaptive rise in PDG activity but did not influence the ammonia excretion response to acute acid loading. Thus, under these experimental conditions the adaptive change in PDG activity was not necessary for the increased ammonia excretion in adult rats during the first 24 hr following an acid load. To fully understand the relationship between the adaptive rises in PDG activity and ammonia excretion, however, other experimental conditions are necessary in which the dissociation between enzyme activity and ammonia excretion might be observed.

The approach taken in the present experiments permits the adaptive rise in PDG activity to occur prior to observing the ammonia excretion response to an acute acid load. It was reasoned that a greater ammonia excretion would be observed in response to the same acid load in an animal with high PDG activity than in an animal with normal enzyme activity. The ammonia excretion response to an acute acid load in rats with high PDG activity is presented here.

Materials and methods. Ammonia excretion rate and phosphate-dependent glutaminase activity in response to chronic acid challenge. Adult, female, Sprague–Dawley rats weighing 175–250 g were used in all experiments. Since an animal with high renal cortical PDG activity was desired, it was necessary to observe the adaptive rise in both enzyme activity and ammonia excretion during and following chronic acid challenge. This was accomplished by placing animals in individual metabolic cages for the collection of urine under oil and replacing drinking water with 1.5% NH_4Cl for 6 days. At the end of 6 days of exposure to this solution, the animals were allowed access to regular drinking water and the rate of fall in both ammonia excretion and PDG activity was followed for the next nine days. Twenty-four-hour ammonia excretion rate was measured on Days 0–9, 13, and 15. Phosphate-dependent glutaminase activity was measured on Days 0, 1, 3, 4, 6, 7, 9, 11, 13, and 15.

To compare the response to an acid challenge in control rats (normal PDG activity) with "adapted" rats (elevated PDG activity), ammonia excretion rate had to be reduced to normal level in the "adapted" animals. This was accomplished as follows: A group of animals was allowed access to 1.5% NH_4Cl drinking water for 6 days. In one-half of this group, NH_4Cl was replaced by regular drinking water for the remainder of the experimental period. The other half was allowed access to 1.5% $NaHCO_3$ for 24 hr following 6 days of NH_4Cl exposure after which the $NaHCO_3$ was replaced with regular drinking water. Phosphate-dependent glutaminase activity

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was measured at the end of the $NaHCO_3$ exposure and ammonia excretion rate was measured for 3 days following termination of the NH_4Cl exposure.

Acute acid load administration. Rats were placed in individual metabolic cages and urine was collected under oil for a 4-hr period. Animals then received 20 mmole NH_4Cl/kg body wt in a constant volume of 3 ml by stomach tube. The animals were returned to their cages and urine was collected under oil every 4 hr for 24 hr and then again at 48 hr following the acid load. A similar procedure was used for "adapted" rats that had been drinking 1.5% NH_4Cl for 6 days followed by 1.5% $NaHCO_3$ for 1 day. Ammonia excretion was measured in each of the 4 hr collection periods and PDG activity was measured at the end of the experimental period.

Analytical techniques. Urinary ammonia was measured by the microdiffusion method of Conway (5). Phosphate-dependent glutaminase activity was measured in renal cortical homogenates by the method of DeBruin and Mattenheimer (6) using freshly prepared L-glutamine as substrate. The PDG activity was determined from the difference in amount of ammonia formed between the experimental dish and the blank which did not contain phosphate. Protein was determined by the biuret method of Robinson and Hogden (7).

All statistical analyses were performed using Student's *t* test.

Results. Chronic metabolic acidosis. Figure 1 shows the response of both PDG activity and rate of ammonia excretion during and following chronic metabolic acidosis induced by 6-day exposure to 1.5% NH_4Cl solution in the drinking water. Phosphate-dependent glutaminase activity did not increase significantly during the first 24 hr of acidosis; however, the ammonia excretion rate did increase during this period from a control value of 6.4 ± 0.7 to 18.9 ± 2.3 μmole NH_3/100 g body wt/hr. Phosphate-dependent glutaminase activity did rise from a control value of 4.32 ± 0.28 μmole NH_3/g cortical protein/hr to a peak value of 11.98 ± 1.10 μmole NH_3/g cortical protein/hr by Day 6. The maximum rate of ammonia excretion of 65.3 ± 7.4 μmole NH_3/100 g body weight/hr, however, was reached on Day 4, 2 days before the peak in PDG activity.

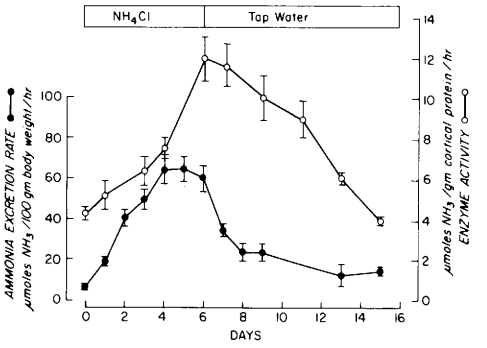

FIG. 1. Ammonia excretion (solid circles) and phosphate-dependent glutaminase activity (open circles) in rats during and following access to 1.5% NH_4Cl drinking water. Values represent mean ± SEM of four to seven animals.

On Day 7, the first day following the removal of the 1.5% NH_4Cl drinking water, the rate of ammonia excretion fell precipitously to 35.1 ± 2.9 μmole NH_3/100 g body wt/hr. However, by the 9th day after removal of the NH_4Cl, the rate of ammonia excretion was still twice as high as the control levels. Phosphate-dependent glutaminase activity fell steadily and by 9 days after the removal of NH_4Cl, enzyme activity was not significantly different from the control value.

Effect of sodium bicarbonate on the rate of ammonia excretion and phosphate-dependent glutaminase activity. The result of access to 1.5% NH_4Cl for 6 days followed by 24-hr access to 1.5% $NaHCO_3$ on ammonia excretion rate is shown in Fig. 2. During the 24 hr of $NaHCO_3$ administration, the rate of ammonia excretion fell from 61.2 ± 5.8 to 9.8 ± 0.4 μmole NH_3/100 g body wt/hr; this latter value was not significantly different from the control level of 6.4 ± 0.7 μmole NH_3/100 g body wt/hr. In the following 2 days, the rate of ammonia excretion fell slightly, but was not significantly different from control levels. Phosphate-dependent glutaminase activity did not fall during the 24 hr of $NaHCO_3$ administration; 11.79 ± 0.82 after $NaHCO_3$ vs 11.93 ± 1.10 μmole NH_3/g cortical protein/hr before $NaHCO_3$.

Response to an acute acid load. Figure 3 shows the comparison of ammonia excretion rate over a 48-hr period between control rats that did not receive an acute acid load and two groups of rats, normal acidotic and

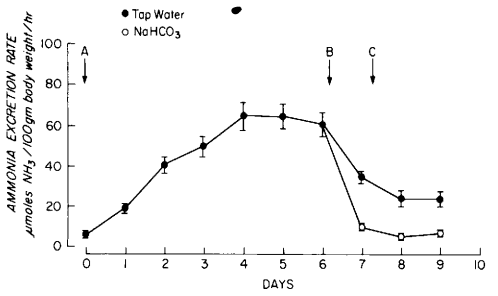

FIG. 2. Ammonia excretion from rats allowed access to 1.5% NH_4Cl drinking water (point A) for 6 days followed (point B) by access to either 1.5% $NaHCO_3$ (open circles) or tap water (closed circles). Point C indicates replacement of 1.5% $NaHCO_3$ with tap water. Values represent mean ± SEM of four to eight animals.

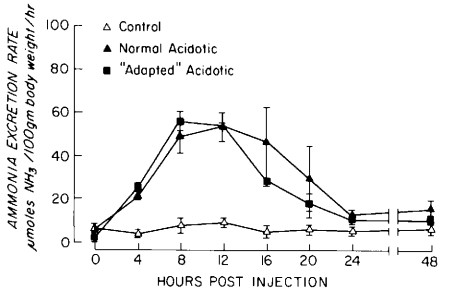

FIG. 3. Ammonia excretion in control animals receiving distilled water (open triangles) or normal acidotic (low PDG activity, solid triangles) and "adapted" acidotic (high PDG activity, solid squares) rats receiving 20 mmole NH_4Cl/kg body wt by stomach tube. Values represent mean ± SEM of 4-hr urine collections from four to seven animals.

"adapted" acidotic, given 20 mmole NH_4Cl/kg body wt by gavage. "Adapted" acidotic rats were prepared as above, 6 days access to 1.5% NH_4Cl followed by 24-hr access to 1.5% $NaHCO_3$. The acute acid load was administered at the end of this period. Since neither the control nor the normal acidotic rats had previous exposure to NH_4Cl, phosphate-dependent glutaminase activity was the same in both groups prior to the administration of the acute acid load, 4.32 ± 0.28 μmole NH_3/g cortical protein/hr. However, phosphate-dependent glutaminase activity in the "adapted" acidotic rats was significantly elevated, 11.98 ± 1.23 ($P < 0.01$), prior to administration of the acute acid load. A small diurnal pattern in ammonia excretion rate was observed in nonacidotic control rats. Except for the initial zero-hr values where ammonia excretion rate was significantly lower in the "adapted" acidotic animals than normal acidotic rats, 2.1 ± 0.3 vs 5.8 ± 0.2 μmole NH_3/100 g body wt/hr, respectively, no significant difference was observed throughout the remainder of the 48-hr period. In dose–response studies, a maximum ammonia excretion rate was achieved following administration of 40 mmole NH_4Cl/kg body wt and was significantly different from the greatest rate following administration of 20 mmole NH_4Cl/kg body wt, 80.8 ± 14.9 vs 53.6 ± 6.1 μmole NH_3/100 g body wt/hr, respectively.

Discussion. Although phosphate-dependent glutaminase (PDG) activity and ammonia excretion rate, in general, rise and fall in parallel fashion (Fig. 1), a direct cause–effect relationship between these two parameters cannot be assumed. Examples of divergence between the two exist. For instance, during the first 24 hr of NH_4Cl administration, ammonia excretion rate rose threefold without a concomitant change in enzyme activity. Also, during the first 24 hr following cessation of NH_4Cl administration, ammonia excretion fell to approximately 50% of its plateau value, again without a significant change in enzyme activity. Although these two situations represent dissociation of ammonia excretion and PDG activity, derepression and repression, respectively, of existing enzyme activity could account for the observed phenomena (8, 9).

In order to test the hypothesis that elevated level of PDG enhances ammonia excretion response to acute acid challenge, an animal model with high renal PDG activity but low ammonia excretion rate had to be developed. Figure 2 demonstrates that ingestion of 1.5% $NaHCO_3$ for 24 hr following several days of acid administration reduced ammonia excretion to control levels; however, PDG activity remained at the elevated level developed during acidosis. The fact that ammonia excretion remained at control levels once the 1.5% $NaHCO_3$ was removed suggests that the previous acid load had been neutralized. Thus, except for the high PDG activity, the "adapted" animals appeared to be similar to control animals.

Figure 3 shows that "adapted" rats did not respond better than control rats to the same acute acid load. This observation suggests that elevation of PDG activity does not en-

hance renal response to acute acidosis. Clearly, this represents a dissociation between ammonia excretion and PDG activity. Other examples of dissociation between these two parameters during acute acid challenge have been reported. Goldstein (4) demonstrated in adult acidotic rats that actinomycin D administration prevented the rise in PDG activity without influencing ammonia excretion. Rector and Orloff (10) have also demonstrated elevation in ammonia excretion without a rise in PDG activity in dogs.

Although it does not appear that elevation of PDG activity is an important factor in renal ammonia excretion response to acute acid loads, the role of the adaptive increase in enzyme activity during chronic metabolic acidosis remains to be determined.

Summary. The role of elevated renal phosphate-dependent glutaminase activity in enhancing ammonia excretion in response to an acute acid load was evaluated in adult rats. Although enzyme activity was approximately threefold higher in "adapted" rats, ammonia excretion rate during the 24 hr following administration of an acute acid load was not significantly greater than from rats with normal enzyme activity. This observation represents an example of dissociation between elevated phosphate-dependent glutaminase activity and ammonia excretion response to an acute acid load.

1. Lotspeich, W. D., and Pitts, R. F., J. Biol. Chem. **168,** 611 (1947).
2. Davies, B. M. A., and Yudkin, J., Biochem. J. **52,** 407 (1952).
3. Rector, F. C., Seldin, D. W., and Copenhaver, J. H., J. Clin. Invest. **34,** 20 (1955).
4. Goldstein, L., Nature (London) **205,** 1330 (1965).
5. Conway, E. J., "Microdiffusion Analysis and Volumetric Error." Crosby, Lockwood, London (1957).
6. DeBruin, H., and Mattenheimer, H., Enzymol. Biol. Clin. **4,** 65 (1964).
7. Robinson, H. W., and Hogden, C. G., J. Biol. Chem. **135,** 707 (1940).
8. Katunuma, N., Tomino, I., and Nishino, N., Biochem. Biophys. Res. Commun. **22,** 321 (1966).
9. Kvamme, E., Tveit, B., and Svenneby, G., Biochem. Biophys. Res. Commun. **20,** 566 (1965).
10. Rector, F. C., and Orloff, J., Fed. Proc. **17,** 129 (1958).

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