PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 64-66 (1976)

Ammonia Formation from Glutamine Isomers by Nonacidotic and Acidotic Perfused Rat Kidneys (39329)

THOMAS C. WELBOURNE

Department of Medicine, University of Montreal, and Clinical Research Institute of Montreal, Montreal, Quebec, Canada

Glutamine-dependent renal ammonia production arises from a dual enzyme system, cytoplasmic D-glutamyltransferase (EC 2.3.2.1), and mitochondrial glutaminase I (EC 3.5.1.2), whose activities subserve different renal functions (1, 2). Besides being situated in separate cellular compartments (2), the two pathways can be differentiated by the fact that the mitochondrial glutaminase is highly stereospecific, acting only upon the L-isomer (3), while the cytoplasmic glutamyltransferase acts on either the L- or D-isomer (2, 4). I therefore employed these two isomers in order to demonstrate the relative activities of the two pathways in nonacidotic and chronically acidotic rat kidneys.

Materials and methods. Kidneys from nonacidotic and NH_4Cl-induced chronic acidotic (5) male Sprague–Dawley rats were isolated and perfused as previously described (6, 7). The perfusion solution consisted of an artificial plasma media with Dextran 110 in place of albumen providing glutamine as the sole source of exogenous nitrogen in these studies. Glutamine was employed at initial concentrations listed in the tables; the purity of commercially supplied (Sigma) D-glutamine was determined after exposure to *Escherichia coli* glutaminase and the liberated ammonia measured. With the five different lots supplied, apparent contamination by L-glutamine was always less than 4% (Sigma considers D-glutamine pure within the limits of their analysis). Perfusing kidneys with 0.04 m*M* L-glutamine, the maximal L-isomer present when perfusing with 1 m*M* D-glutamine, failed to increase ammonia production above those levels observed in the absence of exogenous glutamine. Perfusate ammonia concentrations were measured at 15-min intervals throughout the 60-min perfusion period using a modification of the glutamate dehydrogenase NADH-NAD linked reaction (8). Glutamine concentrations, both L- and D-isomers, were measured by analysis of L-glutamate after L-glutamine hydrolysis with *E. coli* glutaminase (9) or, in the case of D-glutamine, measuring ammonia formation after acid hydrolysis (10). Ammonia concentrations rose linearly during the 60 min studied. Rates of glutamine uptake and ammonia production were calculated (9) and expressed as micromoles per hour per kidney.

Results. Table I presents ammonia production by nonacidotic kidneys perfused with either L- or D-glutamine. At physiological 1-m*M* glutamine concentrations, ammonia produced from D-glutamine was 70% of that from the L-isomer reflecting the small mitochondrial glutaminase I activity which normally contributes only 30% or less, of the total ammonia produced (1, 2, 10). The ratio of ammonia produced per glutamine extracted for D-glutamine was 0.96 reflecting the hydrolysis of the amide nitrogen and transfer of the γ-glutamyl moiety (1) while that for L-glutamine was 1.36 reflecting the mixture of the glutamyltransferase pathway (NH_3/gln = 1.0) and the small mitochondrial glutaminase pathway activity (NH_3/gln = 2.0). As the glutamine concentration is increased, ammonia production from both isomers rises in a linear fashion as a result of cytoplasmic glutamyltransferase activity; this is also indicated by the decrease in the NH_3/gln ratio from 0.96 to 0.85 for D-glutamine and from glutamine serving as both a γ-glutamyl acceptor and donor in the glutamyltransferase reaction (4).

The slight increase in the NH_3/gln ratio from 2.5 to 5.0 m*M* D-glutamine may reflect a contribution of the mitochondrial L-glutamine-utilizing pathway since 5 m*M* D-glutamine would be expected to contain approx 0.2 m*M* L-glutamine although the rise does not achieve statistical significance.

The separate activity of both glutamine-

TABLE I. AMMONIA PRODUCTION FROM GLUTAMINE ISOMERS BY NONACIDOTIC RAT KIDNEYS.

Glutamine concentrations (m*M*)	Ammonia production (μmole hr^{-1})	Glutamine uptake (μmole hr^{-1})	Ammonia / Glutamine
D-Gln			
1 (4)[a]	16.2[b] ± 3.6	16.8 ± 4.3	0.96
2.5 (4)	21.6 ± 4.2	27.0 ± 5.7	0.80
5.0 (4)	37.8 ± 6.9	44.3 ± 7.5	0.85
L-Gln			
1 (4)	20.4 ± 2.0	15.0 ± 3.8	1.36
2.5 (4)	27.6 ± 4.6	23.4 ± 5.1	1.18
5.0 (5)	36.6 ± 8.4	37.8 ± 6.6	0.97

[a] Numbers in parentheses represent perfused kidneys.
[b] Mean ± SE.

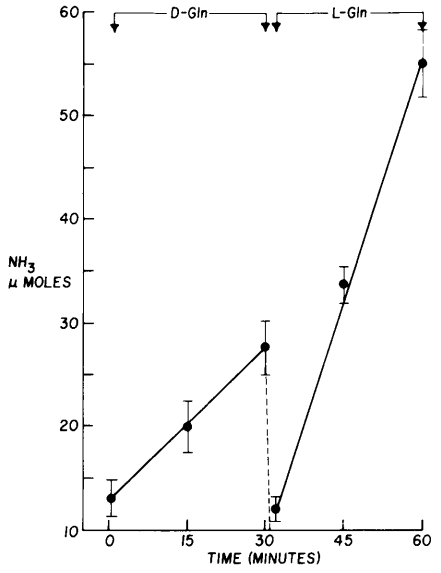

FIG. 1. Perfusate ammonia content during perfusion of four acidotic kidneys with 1 m*M* D-gln for 30 min and then switched to a fresh media of 1 m*M* L-gln for a subsequent 30 min.

utilizing systems, cytoplasmic glutamyltransferase and mitochondrial glutaminase I, can be clearly seen in the same kidney as shown in Fig. 1. Acidotic kidneys are perfused with D-glutamine which penetrates the mitochondrial inner membrane in acidosis (1) but because of specificity restrictions, cannot be hydrolyzed by matrix-located glutaminase I, and as a consequence ammonia is contributed solely by the cytoplasmic pathway. After 30 min the perfusion media is switched, by means of a 3-way stopcock, to a fresh perfusion solution containing L-glutamine and ammonia production then is followed for a subsequent 30 min. Switching from D- to L-glutamine reveals the striking duality of the cytoplasmic and mitochondrial pathways in the acidotic kidney. Ammonia production by the cytoplasmic pathway increased about twofold 16.2 μmole hr^{-1} (Table I) to 29.4 μmole hr^{-1} (Table II), a rise in activity which is consistent with previous observations (9–11).

Ammonia production by the mitochondrial pathway rose from 4.2 μmole hr^{-1} (the difference between L- and D-glutamine's ammonia production, Table I) to 84.7 μmole hr^{-1} (Table II) giving an amazing 20-fold activation as previously observed (1, 12). Apparently, under these conditions, the mitochondrial pathway successfully competes for L-glutamine since the NH_3/gln ratio of 2.0 indicates complete deamidation and deamination of glutamine (13–15). These are the highest ratios I have observed and are probably the result of depriving this pathway of substrate during the perfusion with D-glutamine and the elevated glutaminase I-glutamine utilization rate.

Discussion. The purpose of this report was to demonstrate the existence of the glutamyltransferase and glutaminase I-gluta-

TABLE II. AMMONIA PRODUCTION BY ACIDOTIC KIDNEYS PERFUSED WITH D-GLUTAMINE (0–30 MIN) AND L-GLUTAMINE (32–62 MIN).

Glutamine concentration (mm)	Ammonia production (μmole hr^{-1})	Glutamine uptake (μmole hr^{-1})	Ammonia / Glutamine
D-Gln			
1 (4)[a]	29.4 ± 4.8	30.6 ± 3.6	0.96 ± 0.06
L-Gln			
1 (4)	84.7 ± 6.6	42.5 ± 2.4	1.99 ± 0.06

[a] Numbers in parentheses represent perfused kidneys.

mine utilizing pathways from the perspective of substrate specificity. In addition, the quantitative contribution of each pathway to the total renal ammonia production can be estimated in the normal and chronically acidotic kidneys. The present studies, in agreement with others (1, 2, 10), support the interpretation that glutamyltransferase is the major producer of ammonia in the normal acid-base state, while a 15- to 20-fold activation of the mitochondrial pathway (1, 12) makes it the predominant source of ammonia in acidosis as previously observed (16, 17). The existence of dual pathways has been substantiated from the perspective of nitrogen (1, 7) and carbon (1, in preparation) end product analysis, selective inhibition of one, glutamyltransferase (2, 9, 10), or the other, glutaminase I (11), and by stimulating preferentially one pathway, glutaminase I in acidosis (16), or the other, glutamyltransferase by amino acid loading (18). All of these diverse experimental approaches have supported the major conclusion of the present study.

Summary. The following points summarize these findings: (i) there are 2 glutamine utilizing enzyme systems in the rat kidney; (ii) the cytoplasmic glutamyltransferase system hydrolyzes either glutamine isomer while the mitochondrial localized glutaminase 1 is specific for the L-isomer; (iii) the cytoplasmic pathway contributes 70% of the total renal ammonia production in the normal kidney; (iv) chronic metabolic acidosis results in a 20-fold activation of the mitochondrial glutaminase 1 pathway.

I thank Dr. Jacques Genest, Scientific Director, Clinical Research Institute, for providing me with facilities which made this study possible. The excellent technical assistance of Mrs. Clara Welbourne and Mrs. Grace Thornely-Brown are gratefully acknowledged.

1. Welbourne, T. C., Med. Clin. North. Amer. **59,** 629 (1975).
2. Phenix, P., and Welbourne, T. C., Amer. J. Physiol. **228,** 1269 (1975).
3. Klingman, J. D., and Handler, P., J. Biol. Chem. **232,** 369 (1958).
4. Dixon, M., and Webb, E. C., *in* "Enzymes," p. 707. London, Longman and Green (1964).
5. Welbourne, T. C., Amer. J. Physiol. **224,** 796 (1973).
6. Welbourne, T. C., Canad. J. Physiol. Pharm. **50,** 883 (1972).
7. Welbourne, T. C., Amer. J. Physiol. **226,** 544 (1974).
8. Tabor, C. W., *in* "Methods in Enzymology" (S.P. Colowick and N.O. Kaplan, eds.), Vol. 17, p. 137. Academic Press, New York (1974).
9. Welbourne, T. C., Amer. J. Physiol. **226,** 549 (1974).
10. Wadoux, P., and Welbourne, T. C., Canad. J. Biochem. **53,** 930 (1975).
11. Welbourne, T. C., Amer. J. Physiol. **226,** 555 (1974).
12. Curthoys, N., and Lowry, O., J. Biol. Chem. **248,** 162 (1974).
13. Hird, F. J. R., and Marginson, M. A., **127,** 718 (1968).
14. Pitts, R. F., Pilkington, L. A., and DeHaas, J., J. Clin. Invest. **44,** 731 (1965).
15. Balagura-Baruch, S., *in* "The Kidney" (C. Roniller, and A. F. Muller, eds.), Vol. 3, p. 253. Academic Press, New York (1971).
16. Damian, A. C., and Pitts, R. F., Amer. J. Physiol. **218,** 1249 (1970).
17. Goldstein, L., Amer. J. Physiol. **213,** 983 (1967).
18. Welbourne, T. C., Abs. Canad. Physiol. Soc. St. Sauver, Québec (1975).

Received December 1, 1975. P.S.E.B.M. 1976, Vol. 152.