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Glutaminase-γ-Glutamyltransferase: Subcellular Localization and Ammonia Production in Acidosis (40335)

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In the rat kidney, glutamine is utilized by either the mitochondrial glutaminase 1-glutamate dehydrogenase pathway (1–3) or a glutaminase-γ-glutamyltransferase pathway (4–6). The subcellular location of the glutaminase-γ-glutamyltransferase is unknown, although if localized in the cytosol, it would lend support to the previously proposed hypothesis (7) postulating glutamine utilization by dual pathways. The present study was designed (i) to determine its subcellular location and (ii) to determine its quantitative contribution to ammonia production by acidotic kidneys perfused with 1 m*M* L-glutamine.

Materials and Methods. Male Sprague–Dawley rats, weighing between 350 and 400 g, were tube fed 1400 μmoles of NH_4Cl (0.40 *M*) per day for 3 days; at the end of the second day they were placed in metabolic cages, one per cage, and 24-hr urine collections were observed. Throughout the study the animals were maintained on rat chow (Purina) and water *ad libitum*. A constant ingestion of NH_4Cl ensured a similar degree of acidosis in all rats; this was confirmed by monitoring systemic blood pH and HCO_3 concentration (Radiometer pH–bloodgas analyzer) at time of perfusion and determining 24-hr ammonium excretion.

Two hours prior to perfusion, rats were injected with either methionine-DL-sulfoximine (Sigma), 1.8 mmoles kg^{-1}, ip, dissolved in 1.0 ml of 0.9% saline or 0.9% saline alone. The animals were anesthesized with sodium pentobarbital, 30 mg kg^{-1}, ip, and their kidneys were isolated (8, 9) and perfused with an artificial plasma solution containing albumin (Sigma, fraction IV) and 1 m*M* L-glutamine; albumin was defatted (10) and dialyzed, two changes, for 48 hr against 4 liters of the perfusate solution, minus albumin. Kidneys were perfused, pH 7.40, with 80 ml of perfusate for 60 min and samples of the perfusate media were taken at 15-min intervals. The media were analyzed for ammonia concentration by both the enzymatic (6) and the Conway microdiffusion methods, modified for blood ammonia (4); glutamine concentration was determined by measuring liberated ammonia after enzymatic (*Escherichia coli* glutaminase, Sigma) deamidation (3, 9). Ammonia production and glutamine uptake rates were calculated as described (3, 9).

Following perfusion, acidotic and acidotic plus MSO-treated rat kidneys were homogenized in ice-cold 0.44 *M* sucrose containing 50 m*M* $MgCl_2$ and 2 m*M* HEPES, pH 7.4. Subcellular fractionation was carried out according to a standard schedule (11) on a Sorval RC2B refrigerated, 0–4°, centrifuge; the postmitochondrial fraction was transferred to a Beckman L ultracentrifuge and centrifuged at 105,000 *g* for 1 hr. The fractions obtained, nuclear + cellular debris, mitochondrial, microsomal, and soluble, were resuspended in fresh homogenizing solution and suitable aliquots were assayed for NH_3 and glutamohydroxamate formation by the γ-glutamyltransferase reaction (5). Protein content was determined using the biuret reaction (12) employing bovine albumin (Sigma, fraction IV) as the standard.

Results. The response to the standard NH_4Cl load is shown in Table I. Both groups, control and pre-MSO-treated rats, received an identical acid load, exhibited a similar degree of mild acidosis, and excreted identical amounts of ammonium (coefficient of variation, 6.2 for control and 4.5 for pre-MSO-treated rats). Differences in ammonia production by perfused kidney from MSO-injected rats are not, therefore, due to a variable response to the acid load.

The effect of MSO on ammonia release and glutamine uptake is presented in Table II. Kidneys released 50 ± 4 and 48 ± 6 μmoles of ammonia g^{-1} hr^{-1} in the absence of exogenous glutamine. In the presence of gluta-

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TABLE I. AMMONIUM CHLORIDE INTAKE, SYSTEMIC ACID–BASE BALANCE, AND AMMONIUM EXCRETION.

Pretreated		Intake[a] NH_4Cl (μmoles day^{-1})	Blood pH (U)	Blood HCO_3^- (mEq $liter^{-1}$)	Excreted NH_4^+ (μmoles day^{-1})
Group (4)	1[b]	1400	7.32[c] ±0.06	23.4 ±1.5	1358 ±85
Group (4)[d]	2	1400	7.34 ±0.07	22.8 ±0.9	1375 ±62

[a] Given as 0.4 *M* NH_4Cl, 1400 μmoles day^{-1} for 3 days (see Methods).
[b] To be administered, 0.9% NaCl, 1 ml, ip.
[c] Means ± SEM from four rats.
[d] To be administered, 0.9% NaCl plus MSO, 1.8 mmoles kg^{-1}.

TABLE II. THE EFFECT OF MSO ADMINISTRATION ON AMMONIA RELEASE AND GLUTAMINE UPTAKE.

	Ammonia released (μmoles g^{-1} hr^{-1})	Glutamine uptake (μmoles g^{-1} hr^{-1})	Ammonia/ glutamine
Control[a]			
0 m*M* (4)[b]	50 ± 4	—	
1 m*M* (4)	241 ± 24	119 ± 13	2.02
Δ[c]	191 ± 18	119 ± 13	1.60[e]
MSO[d]			
0 m*M* (4)	48 ± 6	—	2.86
1 m*M* (4)	157 ± 15	55 ± 9	1.98[e]
Δ[c]	109 ± 11[e]	55 ± 9[e]	

[a] Control acidotic rats (Table I).
[b] Number of rats.
[c] Ammonia released with 1 m*M* glutamine–0 m*M* glutamine.
[d] MSO, 1.8 mmoles kg^{-1} given 2 hr prior to perfusion.
[e] Significantly different from control ($P < 0.05$).

mine, control acidotic kidneys released 241 ± 24 μmoles of ammonia while MSO-treated rats released significantly less ($P < 0.01$), 157 ± 15 μmoles of ammonium. Glutamine uptake for acidotic controls was 119 ± 13 μmoles, which fell to only 55 ± 9 μmoles ($P < 0.001$). If one assumes ammonia released, in these studies, accurately reflects production, then the NH_3 produced per glutamine extracted ratios are 2.02 ± 0.05 for acidotic control, and 2.85 ± 0.08 for acidotic plus MSO-treated kidneys. Since a value of 2.0 is the highest possibly attained from complete deamidation and deamination of glutamine, it is clear that ammonia released in the absence of glutamine contributes to the total released in the presence of glutamine. Subtracting this gives an NH_3/Gln ratio of 1.60 in the acidotic control and 1.98 in the MSO-treated acidotic rats.

A direct effect of MSO on ammonia production from glutamine can be shown by adding the inhibitor to the perfusate (Fig. 1). Over the 30-min control period, the production rate averaged 1.43 μmoles min^{-1}; within 10 min production rates fell to 1.04 μmol min^{-1}. The fall in glutamine uptake was disproportionately greater than with ammonia production, falling from 25.9 ± 3.2 μmoles per 30 min to 16.0 ± 2.9 μmoles per min ($P < 0.05$). Consequently, the ammonia produced per glutamine extraction ratio rose from 1.66 to 1.95.

The subcellular localization of the glutamine-utilizing enzyme is shown in Table III. The activity, measured as both a glutaminase (ammonia liberated in absence of NH_2OH) and γ-glutamyltransferase, appears to be a soluble enzyme for the following reasons. The activity is mainly in the soluble fraction, 56% of the total homogenate activity, and its specific activity is significantly enriched only in

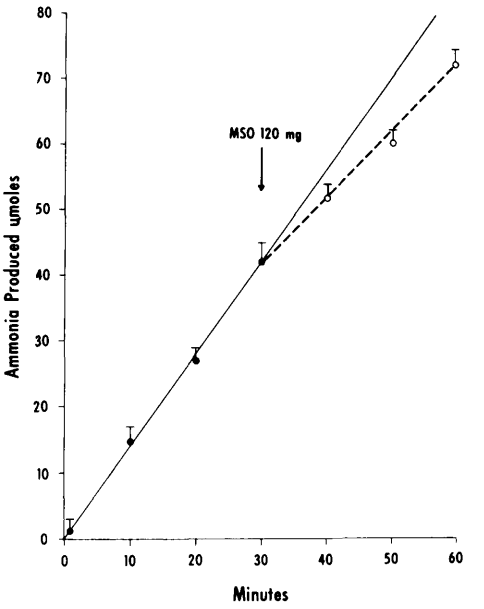

FIG. 1. Acute effect of methionine-DL-sulfoximine, 120 mg in 1.0 ml of 0.9% saline, on ammonia production from glutamine. Results are from four rats given the standard acid load (see Methods). Ammonia produced represents the total released minus the amount released in the absence of glutamine. Kidneys were perfused with 80 ml of perfusate; production rate is linear over the 60-min period in the absence of MSO.

TABLE III. RENAL GLUTAMINASE-γ-GLUTAMYLTRANSFERASE: SUBCELLULAR LOCALIZATION AND MSO INHIBITION.

	Ammonia[a]		γ-GHA[b]	
Fraction	Total activity[c]	S.A.[d]	Total activity	S.A.
Homogenate[e]				
Control	215 ± 16	1.5 ± 0.1	860 ± 45	6.0 ± 0.3
MSO	18 ± 5	0.1 ± 0.0	83 ± 8	0.6 ± 0.1
Nuclear				
Control	57 ± 8	1.8 ± 0.2	146 ± 13	4.6 ± 0.5
MSO	19 ± 11	0.6 ± 0.4	12 ± 3	0.4 ± 0.1
Mitochondrial				
Control	16 ± 5	0.3 ± 0.1	69 ± 14	1.4 ± 0.3
MSO	10 ± 7	0.2 ± 0.1	9 ± 3	0.2 ± 0.1
Microsomal				
Control	47 ± 6	2.2 ± 0.3	160 ± 21	7.4 ± 0.9
MSO	9 ± 4	0.4 ± 0.2	25 ± 12	1.0 ± 0.5
Soluble				
Control	98 ± 12	2.8 ± 0.3	481 ± 33	13.8 ± 1.0
MSO	6 ± 3	0.1 ± 0.1	19 ± 5	0.5 ± 0.1

[a] Ammonia produced in the absence of NH_2OH.
[b] γ-Glutamohydroxamate formed in the presence of NH_2OH.
[c] Total activity, μmoles hr^{-1}, per fraction.
[d] Specific activity, μmoles hr^{-1}, per mg of protein.
[e] Mean ± SEM from four kidneys in each group.

the soluble fraction. Both ammonia production and γ-GHA formation were markedly inhibited in the soluble fraction to values less than 15% of the control. Noteworthy ammonia production by the mitochondrial fraction (glutaminase 1 pathway) was unaffected.

Discussion. The results clearly demonstrate the inhibition of a glutaminase-γ-glutamyltransferase activity localized in the soluble fraction (Table III) which contributes 30 to 40% of ammonia produced by these mildly acidotic kidneys (Table II and Fig. 1). The disproportionately greater fall in glutamine uptake, 54%, than in ammonia production, 43%, is consistent with the glutaminase-γ-glutamyltransferase pathway contributing only one ammonia per glutamine. The rise in the NH_3 produced/glutamine extracted ratio to 2.0 after inhibition of the cytoplasmic pathway is consistent with complete deamidation and deamination by the mitochondrial pathway. These results therefore support the previous proposal of dual glutamine-utilizing pathways in the rat kidney with NH_3/Gln ratios reflective of the contribution from each pathway.

The present study underlines an important point in calculating the ammonia produced to glutamine extraction ratio (Table II). It must be realized that total ammonia release is not necessarily equivalent to that produced from the glutamine extracted. Thus, Hems (13) observed that nonacidotic kidneys perfused with 1 m*M* L-glutamine released 119 μmoles of NH_3 per 45 μmoles of glutamine, giving an NH_3/Gln ratio of 2.64; ammonia released in the absence of glutamine was similar to the present study, some 47 ± 4 μmoles. Since a ratio of greater than 2 is clearly impossible, subtracting the glutamine-independent release, 47 μmoles, from 119 gives 72 actually produced from glutamine and an NH_3/Gln ratio of 1.6. Ross (14) calculated an ammonia recovered to glutamine removed ratio of 1.9 with 1 m*M* L-glutamine as the substrate; however, if 47 μmoles of glutamine-independent ammonia release is subtracted, the ratio falls to 130 − 47 = 83/68 or 1.22; furthermore, subtracting a similar ammonia blank from the ammonia released by acidotic kidneys, 297 − 47 = 250 μmoles, and dividing by glutamine removed, 154 μmoles, gives a ratio of 1.62. In previous work, employing dextran in place of albumin, I observed an ammonia/glutamine ratio of 1.4 in nonacidotic, increasing to 1.8 in acidotic rat kidneys (3, 4, 6). Subsequently, the role of a glutaminase-γ-glutamyltransferase was revealed in a series of studies (5, 6, 7, 15) culminating in the isolation of the enzyme from the soluble fraction (15).

The exact identity of this glutaminase-γ-

glutamyltransferase is at present unclear. It clearly is not γ-glutamyltranspeptidase (5, 15) and is probably not glutamine synthetase (although this enzyme complex is capable of glutaminase-γ-glutamyltransferase activity and is inhibited by MSO) since synthetase is predominantly microsomal (15, 16, 17) while the present activity is predominantly soluble (Table III, 15). Another enzyme, γ-glutamylcysteine synthetase, is a soluble protein (18) and inhibited by MSO (19), but does not utilize glutamine (20). Consequently, further studies are required to determine the exact identity of this activity.

Summary. Glutaminase-γ-glutamyltransferase contributes some 30% of the ammonia produced from glutamine by mildly acidotic rat kidneys. The enzyme is localized in the cytosol and its inhibition results in an ammonia produced per glutamine extracted ratio of 2.0. The results are therefore consistent with a dual glutamine-utilizing system, one cytoplasmic and the other mitochondrial, in the functioning rat kidney.

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