The Metabolic Fate of Glutamine Nitrogen in the Perfused Rat Kidney

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The fate of glutamine amide and amino nitrogen was determined in the perfused rat kidney. There is a deficit of about 40% in the recovery of glutamine nitrogen and NH_3 in the perfusion medium. After correction for the tissue content of glutamine, the remaining nitrogen was fully accounted for by small amounts of NH_3 , glutamate, aspartate and a compound that liberated glutamate on acid hydrolysis, probably 5-oxopyrrolidine-2-carboxylate (pyroglutamate). The latter accounted for 8% of the glutamine metabolized.

Welbourne (1974) and Hems (1972) reported that, in kidneys perfused with 5 mM-glutamine, less NH_3 was recovered than expected; in each case, only approx. 1 mol of NH_3 was found for each 1 mol of glutamine removed. Ross (1976) observed a similar deficit in glutamine nitrogen recovered in the perfusion medium, even after taking account of the amino acids in the samples, although, when the initial concentration of glutamine was 1 mM rather than 5 mM, 2 mol of NH_3 was recovered from each 1 mol of glutamine removed. For a proper understanding of the metabolic potential of the intact kidney, identification of all the products of glutamine removed is desirable. A complete balance of metabolites in perfusion experiments requires the determination of tissue metabolite concentrations [e.g. Ross (1972), and in liver perfusion (Krebs *et al.*, 1973)]. The present paper includes data from the analysis of the kidney tissue, freeze-clamped after perfusion with 5 mM- or 1 mM-glutamine, and a recalculation of the total fate of glutamine nitrogen.

Methods

Kidneys of fed rats were perfused by the method of Ross *et al.* (1973) with 70 ml of perfusion medium containing 1 mM- or 5 mM-glutamine for 30 min, at which time the kidneys were freeze-clamped (Wollenberger *et al.*, 1960). HClO_4 extracts were prepared. Glutamine was determined by the method of Lund (1974), NH_3 by the method of Kun & Kearney (1974), glutamate by the method of Bernt & Bergmeyer (1974a), alanine by the method of Williamson (1974) and aspartate by the method of Bergmeyer *et al.* (1974). For determination of '5-oxopyrrolidine-2-carboxylate', extracts were boiled for 2 h with 2 M-HCl, and the total glutamate concentration was determined; '5-oxopyrrolidine-2-carboxylate' concentration is the

difference between this value and the glutamate-plus-glutamine concentration in non-hydrolysed extracts. No glutamate was liberated from glutathione under these conditions.

Glutathione was determined enzymically (glyoxy-lase) on freshly neutralized extracts (Bernt & Bergmeyer, 1974a,b), with added EDTA (final concn. 0.3 mM) as suggested by Viña *et al.* (1978).

For the determination of metabolites recovered in kidney tissue, the excess in metabolite concentration for kidneys perfused with 5 mM-glutamine over those perfused with 1 mM-glutamine has been used. With kidneys perfused with 1 mM-glutamine, nitrogenous products determined in the medium and in the urine fully accounted for the glutamine removed (see Table 1); it could be assumed, therefore, there would be virtually no increase in glutamine-derived nitrogenous constituents in the kidney tissue itself under these conditions. Use of non-perfused controls, or of controls perfused with another substrate, is unsatisfactory owing to differences in tubular fluid content in the former and 'wash-out' of substrates in the latter. The 'excess' values for the nitrogenous constituents in kidney for the 5 mM-glutamine perfusion were compared with the glutamine removed from the medium not accounted for by products in the perfusion medium.

Results

Rates of metabolic change in the perfusion medium and urine when kidneys of fed rats were perfused with either 1 mM-glutamine or 5 mM-glutamine are shown in Table 1. Within the limits of experimental error, the theoretically expected recovery of 2 mol of NH_3 per mol of glutamine removed was observed when the substrate was 1 mM-glutamine. When 5 mM-glutamine was substrate, only 1.28 mol of NH_3 per mol of glutamine removed appeared in the medium, confirming the earlier observations by Hems (1972) and

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Table 1. *Metabolism of glutamine in perfused kidney of well-fed rat*

Conditions of perfusion as described in the Methods section. Glutamine (5 mM or 1 mM) was added before starting perfusion, and samples of medium were taken for determination of metabolites at 10 min intervals. Rates of change of metabolite content of the medium were calculated. Linear rates of glucose synthesis and NH_3 formation were maintained for at least 60 min. Results are given as $\mu\text{mol}/30 \text{ min per g wet wt.}$ and are means $\pm$ S.E.M. for six perfusions.

Metabolite	Addition ...	5 mM-Glutamine	1 mM-Glutamine
Glucose formed		5.87 ± 0.81	2.2 ± 0.34
NH_3 formed (in medium)		28.5 ± 2.2	13.0 ± 1.6
NH_3 excreted (in urine)		2.39 ± 0.6	0.8 ± 0.2
Glutamine removed		24.0 ± 2.6	6.8 ± 0.9
Glutamate formed		0.74 ± 0.3	0.2 ± 0.4
Lactate formed		0.05 ± 0.11	—
NH_3 recovered/glutamine removed		1.28	2.0
NH_3 unaccounted for		-16.4	0.4

Table 2. *Metabolites of glutamine recovered in perfused rat kidney*

Kidneys of fed rats were perfused for 30 min and freeze-clamped. Results are given as μmol of metabolite/g wet wt. of kidney and are means $\pm$ S.E.M. for five kidneys in each group. 'Total glutamate' recovered after acid hydrolysis of extracts was 13.6 ± 0.9 in perfusions with 5 mM-glutamine, and 4.9 ± 1.0 in perfusions with 1 mM-glutamine.

Metabolite	Conditions ...	5 mM-Glutamine (A)	1 mM-Glutamine (B)	Excess nitrogen metabolite (A-B)
Glutamine		6.6 ± 0.3	1.3 ± 0.2	5.3
Glutamate		3.8 ± 0.7	3.5 ± 0.8	0.3
'5-Oxopyrrolidine-2-carboxylate'		3.2 ± 0.7	0.1 ± 0.1	3.1
Glutathione		0.96 ± 0.04	1.05 ± 0.05	0.0
Aspartate		1.0 ± 0.2	0.6 ± 0.1	0.4
Alanine		0.54 (2)	0.55 (2)	0.0
NH_3		4.4 ± 0.7	2.6 ± 0.2	1.8
Total glutamine nitrogen recovered in kidney*		29.0	13.1	15.9

* Glutamine nitrogen recovered is calculated from the sum of products, allowing for 2 nitrogen atoms/molecule of glutamine and 3 atoms/molecule of glutathione. The 'A-B' value is the relevant excess of glutamine nitrogen present when 5 mM-glutamine rather than 1 mM-glutamine was perfused.

Welbourne (1974). Table 2 shows the tissue concentrations of the relevant glutamine-nitrogen-containing metabolites after 30 min perfusion.

The main difference between the higher and lower concentrations of glutamine are the accumulation of significant concentrations of glutamine, NH_3 and aspartate in the kidney at the higher concentration of glutamine. In addition, an acid-hydrolysable product, probably 5-oxopyrrolidine-2-carboxylate, accumulated in kidneys to a concentration of 3 mM after perfusion with 5 mM-glutamine. No excess of glutamate, alanine or glutathione was found.

Discussion

Glutamine nitrogen unaccounted for in the 5 mM-glutamine perfusions may be calculated from Table 1. Glutamine nitrogen was recovered as NH_3 in medium and urine, and a small amount of glut-

amate in the medium. Since 2 nitrogen atoms/glutamine molecule are expected, $16.4 \mu\text{mol}$ of glutamine nitrogen/g wet wt. of kidney are unaccounted for after 30 min perfusion, when only medium and urine are taken into account.

Previous investigations of the fate of glutamine nitrogen have neglected the kidney content of metabolites. All but 2% of the unaccounted for nitrogen ($15.9 \mu\text{mol}$) is recovered within the kidney (see Table 2, last column), as glutamine itself (65%), as glutamate (2%), as 5-oxopyrrolidine-2-carboxylate or a related hydrolysable product of glutamate (19%), as aspartate (3%) and as intra-renal NH_3 (11%).

As a result, even at 5 mM-glutamine, 1.76 mol of NH_3 appears per 1 mol of glutamine actually metabolized, and the overall loss of glutamine nitrogen is negligible. Some 8% of the glutamine removed in 30 min was recovered in the kidney as 2-oxopyrrolidine-2-carboxylate or a related compound. Whether this was produced by metabolism within the kidney

or by spontaneous degradation of glutamine remains to be established.

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