Measurements of the turnover rate of glutamine in normal and acidotic rats

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The turnover of glutamine was measured in rats with [1-¹⁴C]glutamine. Rates of turnover measured by constant infusion of the label were 130 μ mol/h per 100 g body wt. in control rats and were not changed in chronic metabolic acidosis. These rates of turnover of whole-body glutamine were about 13–26% of the rate of net glutamine release into the bloodstream, indicating that the bulk of glutamine turnover *in vivo* is concerned with intracellular processes rather than inter-organ fluxes.

Glutamine is an important amino acid in the animal body serving both as a nitrogen carrier and as a carbon source in different tissues. Measurements of arteriovenous differences across various organs have given direct information on the blood-borne flux of glutamine. Glutamine is utilized by the intestine and kidney of normal animals and is released by muscle and liver. During metabolic acidosis, the renal utilization of glutamine is greatly increased, so as to provide urinary ammonia. In addition to inter-organ fluxes, it is apparent that glutamine synthesis may occur in tissues without net release of glutamine. For example, in the kidney of normal rats, Damian & Pitts (1970) have demonstrated glutamine synthesis. Lueck & Miller (1970) have shown that the perfused liver is capable of synthesizing and hydrolysing glutamine simultaneously. In brain substantial glutamine synthesis occurs as part of the 4-aminobutyrate cycle (Norenberg & Martinez-Hernandez, 1979).

There is little information on the magnitude of total glutamine synthesis in animals. In the present experiments we have determined total glutamine turnover in normal animals and in acidotic animals, where there is an increased renal demand for glutamine. We were interested in: (a) what is the whole-body turnover of glutamine in the intact rat?; (b) what proportion of this glutamine turnover is due to the uptake of glutamine by the acidotic kidney? To minimize recirculation of ¹⁴C label, [1-¹⁴C]glutamine was synthesized and used in the measurement of turnover rates. This ¹⁴C label is released as ¹⁴CO₂ as a consequence of the action of 2-oxoglutarate dehydrogenase.

Materials and methods

Rats

Male Sprague–Dawley rats weighing 300–400 g were obtained from Canadian Breeding Farms and

Laboratories, St. Constant, La Prairie, Que., Canada. They were given free access to Purina Rat Chow and water. Metabolic acidosis was induced by substitution of 1.5% NH₄Cl for the drinking water for 7–10 days. Food was withheld for 4 h before each experiment to allow the gut to empty of food.

Reagents

Analytical-grade chemicals used were obtained from either J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A., or Fisher Scientific Co., Fair Lawn, NJ, U.S.A. All enzymes were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. L-[1-¹⁴C]Glutamic acid of specific radioactivity 30–50 mCi/mol was obtained from Amersham/Searle Corp., Don Mills, Ont., Canada. L-[1-¹⁴C]Glutamine was prepared and purified as previously described (Squires & Brosnan, 1978).

Experimental procedure

Rats were anaesthetized by intraperitoneal injection of inactin (0.1 ml of 100 mg/ml solution in 0.9% NaCl/100 g body wt.). In the constant-infusion experiments, [1-¹⁴C]glutamine in Krebs–Henseleit bicarbonate saline (Krebs & Henseleit, 1932) was infused via a heparinized catheter (PE50 tubing) into the right external jugular vein by using a constant-infusion pump. After an initial priming dose of 0.7 ml (3–3.5 μ Ci), which was administered in about 5 s, the [1-¹⁴C]glutamate was infused at a constant rate of about 1.9 ml/h for 60–90 min. Samples were taken from a cannula in the left carotid artery at known times for measurement of [1-¹⁴C]glutamine radioactivity. Samples were also taken at the beginning and the end of the infusion for the determination of plasma glutamine concentrations. The withdrawal of samples was timed so that the volume of Krebs–Henseleit bicarbonate saline containing [1-¹⁴C]glutamine infused would replace the

blood volume taken. Throughout the infusion, the rectal temperature was monitored with a thermometer and the rat was warmed to maintain constant body temperature. The respiration rate and blood pH, $p\text{CO}_2$ and $p\text{O}_2$ were determined to ensure that the animal's condition was stable.

The single-injection experiments were performed by using a similar technique. $[1-^{14}\text{C}]$ Glutamine (6.4 μCi) was administered as one bolus (0.6 ml) via the jugular-vein catheter. Krebs–Henseleit bicarbonate saline was continuously infused throughout the course of the experiment at approx. 3.8 ml/h and was timed so as to replace fluid lost in the withdrawal of blood samples from the arterial cannula.

The specific radioactivity in $[1-^{14}\text{C}]$ glutamine was determined as previously described (Squires & Brosnan, 1978). The concentration of glutamine in the plasma samples was determined with an amino acid analyser (Squires *et al.*, 1976).

Calculations

The turnover rate by constant infusion (V_i) is given by

$$V_i = V_{si}/b$$

where V_{si} is the rate of infusion of label (curies/time) and b is the specific radioactivity of the glutamine pool, constant at plateau. The turnover rate by single injection was calculated by using a double-exponential fit, as described by Shipley *et al.* (1967). Differences between turnover rates were tested for statistical significance by Student's *t* test.

Results

A typical set of results obtained from a single-injection experiment is plotted in Fig. 1. After an initial rapidly changing rate of decline in log (plasma glutamine specific radioactivity), a linear rate of decrease was obtained. A typical set of results obtained by using the constant-infusion technique is plotted in Fig. 2. Note that a plateau in the specific radioactivities of plasma glutamine was quickly established.

Fig. 1. Glutamine turnover after a single injection of $[1-^{14}\text{C}]$ glutamine

Shown here is a typical plot of data obtained by the single-injection technique. In this particular experiment, a normal rat (body wt. 302 g) was used. $[1-^{14}\text{C}]$ Glutamine was injected as a bolus of 0.6 ml (6.4 μCi) via the jugular-vein catheter. For full experimental details see the text.

Fig. 2. Plasma glutamine specific radioactivity during constant infusion of $[1-^{14}\text{C}]$ glutamine

The results of a typical constant-infusion experiment are shown. In this experiment an acidotic rat was used (body wt. 322 g). After an initial priming dose of 0.7 ml (2.7 μCi) of $[1-^{14}\text{C}]$ glutamine, label was continuously infused at a rate of 7.3 $\mu\text{Ci}/\text{h}$. For full experimental details see the text.

Table 1. Turnover rates of [$1\text{-}^{14}\text{C}$]glutamine in normal and acidotic rats

For experimental details see the text. Turnover rates are expressed as means $\pm$ S.D., the numbers in parentheses referring to the number of experiments performed in each group. Statistical significance: N.S., not significant.

	Turnover rate ($\mu\text{mol/h}$ per 100 g body wt.)	
	Single-injection technique	Constant-infusion technique
Normal	208.2 $\pm$ 29.1 (4)	133.8 $\pm$ 8.4 (5)
Acidotic	178.2 $\pm$ 32.5 (5)	131.2 $\pm$ 27.4 (5)
P (normal different from acidotic)	N.S.	N.S.

The turnover rates of [$1\text{-}^{14}\text{C}$]glutamine in normal and acidotic rats measured by both techniques are reported in Table 1. There was no statistical difference in turnover rates of glutamine between normal and acidotic rats, as measured by both methods. However, rates measured by the single-injection method were higher than those measured by the constant-infusion technique.

Discussion

The turnover rates of glutamine and other amino acids have been measured in intact animals and in various preparations *in vitro*. Duda & Handler (1958) measured the turnover of glutamine in liver after the injection of [*amido*- ^{15}N]glutamine into the blood. They obtained a value of 2.7 h for the turnover time (i.e. the time it takes for the entire pool to turn over), which, when taken with a measured total body pool of 1.25 mmol, gave a value of 463 $\mu\text{mol/h}$ per 300–400 g rat, equivalent to 120–150 $\mu\text{mol/h}$ per 100 g body wt. for the turnover rate of body glutamine. This value is similar to those reported in Table 1.

The turnover rate of glutamine has previously been measured in sheep by constant infusion of [$U\text{-}^{14}\text{C}$]glutamine (Heitmann & Bergman, 1975). Those investigators determined values of 12.2 $\pm$ 0.7, 13.1 $\pm$ 0.3 and 12.0 $\pm$ 0.9 mmol/h per animal for fed, NH_4Cl -acidotic and 3-day-starved sheep.

Turnover rates for other amino acids have been measured by a number of workers. Waterlow & Stephen (1967) reported values for lysine turnover in the rat of approx. 180 $\mu\text{mol/h}$ per 100 g body wt. They compared this with values of 43, 18, 32 and 66 $\mu\text{mol/h}$ per 100 g body wt. obtained for valine, isoleucine, leucine and glycine respectively by other

workers. Thus many amino acids probably turn over at significantly lower rates than does glutamine.

Of interest is the discrepancy in the value of the turnover rate of glutamine as measured by the two different methods. The turnover rate as measured by the single-injection technique was always substantially greater than that measured by the constant-infusion method. A similar but much smaller discrepancy in the values obtained for the turnover rate of glucose has been reported. Processes that account for the discrepancy in the values obtained for the turnover of any metabolite include mixing of the label throughout the total pool. The total pool will consist of the extracellular space as well as any tissue pool to which label may be accessible. However, any metabolite that is concentrated within only one pool (i.e. the extracellular space) will have its label more quickly and simply dispersed than would one that is spread throughout various tissues. Such rapid dispersion of label will make the curve of log (specific radioactivity) versus time approach the mono-exponential form more quickly and allow a simple interpretation to be applied. Thus, whereas the bulk of the free glucose in the body is in the plasma and interstitial fluids, the extracellular glutamine pool forms less than 1% of the total body glutamine pool. Therefore, although the application of a simplified formula gives only minor differences from the constant-infusion value for glucose, much larger discrepancies can be expected for glutamine. For this reason we consider the constant-infusion method to be a more reliable technique for determining glutamine turnover.

Schrock & Goldstein (1981) have examined inter-organ glutamine metabolism *in vivo* by measuring arteriovenous differences and blood flows through the major organs of the normal and the acidotic rat. Only muscle was found to release glutamine. In the normal rat they observed a net glutamine release from muscle of 0.28 $\mu\text{mol/min}$ per 100 g body wt. This amounts to a new release into the bloodstream of 16.8 $\mu\text{mol/h}$ per 100 g body wt., or about 13% of the turnover observed in Table 1. In the NH_4Cl -acidotic rat the muscle release was 0.57 $\mu\text{mol/min}$ per 100 g body wt. This amounts to 34.2 $\mu\text{mol/h}$ per 100 g body wt., or about 26% of the turnover observed in Table 1. Although other tissues may also have released glutamine into the blood, it is unlikely that they are major compared with the production by muscle and liver. Thus an important conclusion from this study is that intracellular glutamine utilization is a much larger fraction of total body glutamine utilization than is net release into the blood and transport to other tissues. This is perhaps not surprising in view of the many metabolic processes, such as synthesis of purines, pyrimidines, amino sugars etc., that require glutamine. In addition, there is considerable hydrolysis

and resynthesis of glutamine in liver (Lueck & Miller, 1970), kidney (Damian & Pitts, 1970) and brain (Norenberg & Martinez-Hernandez, 1979).

The data of Schrock & Goldstein (1981) indicate that in NH_4Cl -acidosis the rat kidneys extract an additional $25.2 \mu\text{mol/h}$ per 100 g body wt. However, our data indicate no increase in the whole-body turnover of glutamine. These findings are reconciled by the work of Damian & Pitts (1970), who measured the rate of renal glutamine synthesis in normal and acidotic rats. These workers found quite a high rate of renal glutamine synthesis in normal rats, $24.5 \mu\text{mol/h}$ per g of kidney. The renal synthesis of glutamine decreased to about $2.9 \mu\text{mol/h}$ per 100 g body wt. in NH_4Cl -acidotic rats. Thus these values imply a decrease in renal glutamine synthesis of about $16.5 \mu\text{mol/h}$ per 100 g body wt. during metabolic acidosis. This decrease can account for the fact that overall glutamine turnover remains constant during metabolic acidosis despite increased net renal utilization.

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