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The contribution of phenylalanine to tyrosine metabolism *in vivo*

Studies in the post-absorptive and phenylalanine-loaded rat

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1. Rates of appearance and oxidation of plasma L-leucine, L-phenylalanine and L-tyrosine, as well as conversion of plasma phenylalanine into plasma tyrosine, were determined in 90–120g rats after overnight starvation and while receiving 115–120μmol of L-phenylalanine/h. 2. In the post-absorptive state, plasma tyrosine and phenylalanine appearances were similar, despite the fact that 22% of plasma tyrosine appearance could be attributed to the hydroxylation of phenylalanine. 3. A constant infusion of 115–120μmol of L-phenylalanine/h did not significantly alter plasma leucine kinetics, but increased appearance of plasma phenylalanine and tyrosine. The percentage of phenylalanine and tyrosine appearance that was oxidized increased from 12.1% and 24.4% to 37.3% and 48.0% respectively. In phenylalanine-loaded rats, 72% of plasma tyrosine appearance could be attributed to the conversion of phenylalanine. 4. Whole-body tyrosine oxidation measured from a continuous infusion of either L-[^{14}C]tyrosine or L-[^{14}C]phenylalanine differed by 165%. 5. It can be concluded that, in the post-absorptive state, phenylalanine hydroxylation makes a substantial contribution to the plasma appearance of tyrosine and is significantly increased when phenylalanine is administered. The disposal of excess infused phenylalanine is a result of a greater percentage of plasma phenylalanine being converted into tyrosine and a greater proportion of tyrosine being further oxidized. However, apparent tyrosine oxidation rates estimated from plasma tyrosine specific radioactivities and appearance of expired $^{14}CO_2$ during administration of [^{14}C]tyrosine are underestimates of true rates, in part because tyrosine generated from phenylalanine hydroxylation is catabolized without freely equilibrating with the plasma compartment.

The conversion of L-phenylalanine into L-tyrosine involves irreversible oxidation by cytosolic phenylalanine hydroxylase with tetrahydropteridine as the immediate electron donor. As early as 1913, Embden & Baldes (1913) suggested that the hydroxylation of phenylalanine represented the initial step in the oxidative degradation of phenylalanine and biosynthesis of tyrosine. More recent studies (Moss & Schoenheimer, 1941; Brand & Harper, 1974) have confirmed that the hydroxylation of phenylalanine represents the principal pathway for its catabolism, and during periods of dietary tyrosine deprivation provides adequate quantities of tyrosine to sustain growth in the rat (Womack & Rose, 1934) and nitrogen equilibrium in man (Burrill & Schuck, 1964; Talbert & Watts, 1963).

Such a close interaction between a dietary indispensible amino acid and a semi-indispensible one suggests that the kinetics for the two amino acids would be strikingly different from those for other indispensible amino acids. First, because a component of tyrosine appearance is an irreversible step in the oxidation of phenylalanine, a greater fraction of tyrosine appearance is presumed to be catabolized than would be expected with an indispensible amino acid. However, James *et al.* (1976) have previously reported, in well-nourished adults infused with [^{14}C]tyrosine, that the percentage of tyrosine appearance oxidized entirely to CO_2 was 15–20%, which was very similar to the 15–25% reported for [^{14}C]leucine (Golden & Waterlow, 1977) or [^{13}C]leucine (Motil *et al.*, 1981).

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Secondly, the proportion of phenylalanine appearance that would be oxidized entirely to CO_2 is also expected to be considerably less than with other dietary indispensible amino acids, since the principal fate of its oxidative metabolite, tyrosine, is not further degradation but incorporation into whole-body protein. As expected, initial studies (Dalgliesh & Tabechian, 1956) reported that $^{14}CO_2$ production in rats given [^{14}C]phenylalanine was approximately half of that of rats infused with [^{14}C]tyrosine. More recent reports have shown, however, that $^{14}CO_2$ production was only slightly less, but not significantly so, in rats given [^{14}C]phenylalanine than when animals were infused with [^{14}C]-leucine, -lysine or -valine (Neal & Waterlow, 1974).

Work *in vitro* has clearly demonstrated an interaction between phenylalanine and tyrosine metabolism, but only limited attempts have been made to estimate the quantitative importance of phenylalanine hydroxylation to tyrosine metabolism *in vivo*. Those studies that have examined phenylalanine and tyrosine metabolism *in vivo* have generally relied on the clearance of a single bolus administration of phenylalanine, which has necessitated multiexponential curve-fitting in the non-steady state, a not yet fully validated kinetic approach (Curtius *et al.*, 1977; Zagolak *et al.*, 1977; Trefz *et al.*, 1976).

James *et al.* (1976) used a continuous infusion of L-[U-^{14}C]tyrosine in healthy adults, and hypothesized that 15% of plasma tyrosine appearance was derived from phenylalanine. In a report by Clark & Bier (1982), appearance of plasma phenylalanine and tyrosine was measured from the constant infusion of [*ring*-$^2H_5$]phenylalanine and [1-^{13}C]-tyrosine, and these investigators observed in healthy post-absorptive adults that 13–19% of plasma tyrosine appearance was derived from plasma phenylalanine.

However, neither of these two studies evaluated the compartmentalization of free tyrosine nor whether the conversion of phenylalanine into tyrosine *in vivo* was sensitive to phenylalanine administration. The purpose of the present study was to estimate, by using steady-state kinetics, the quantitative importance of phenylalanine metabolism to tyrosine appearance and oxidation in post-absorptive rats and animals receiving an intravenous infusion of L-phenylalanine.

Experimental

Radioisotopes and chemicals

L-[U-^{14}C]Tyrosine (450Ci/mol), L-[1-^{14}C]tyrosine (40Ci/mol), L-[U-^{14}C]phenylalanine (450Ci/mol), L-[2,3-^{3}H]phenylalanine (15Ci/mol), L-[1-^{14}C]leucine (300Ci/mol) and $NaH^{14}CO_3$ (10Ci/mol) were all obtained from New England Nuclear Corp., Boston, MA, U.S.A. Tyrosine decarboxylase (EC 4.1.1.25) and phenylalanine decarboxylase (EC 4.1.1.53), as well as ninhydrin and L-leucyl-L-alanine, were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Injectable-grade L-phenylalanine was obtained from Ajinomoto Co., New York, NY, U.S.A., and was pyrogen-free. Physiological saline (0.9% NaCl) was sterile and pyrogen-free (Abbott Laboratories, N. Chicago, IL, U.S.A.).

Study design

Male New England Deaconess Hospital rats (NEDH/c), a Wistar–Slonegar strain, were obtained from the Cancer Research Institute, New England Deaconess Hospital. Before investigation, the animals were housed, two per unit, in stainless-steel suspension cages in a light- and temperature-controlled room, where they were allowed to consume *ad libitum* a standard laboratory chow (Charles River RMC 3000; Agway Food Products, Syracuse, NY, U.S.A.) and tap water. Rats with a body weight of between 90 and 120g were starved overnight.

The following morning, between 08:00 and 10:00h the rats were gently restrained by wrapping in cloth (Garlick *et al.*, 1973), and a 26-gauge stainless-steel catheter was inserted into the lateral tail vein. The catheter was attached to Silastic tubing [0.011in × 0.024in (0.28mm × 0.61mm); Dow–Corning Laboratories, Corning, NY, U.S.A.] and 2.34–2.36ml of physiological saline/h was infused with a syringe pump (Harvard Apparatus Co., Harvard, MA, U.S.A.).

Five groups of rats were studied. Half of the animals from each group were infused with physiological saline only and the remainder with physiological saline containing 50μmol of L-phenylalanine/ml. In this manner, half of the animals were post-absorptive and infused with only saline, while the other half were post-absorptive but received 115–120μmol of L-phenylalanine/h.

Added to the sterile physiological saline or phenylalanine solutions were L-[1-^{14}C]leucine (group I), or L-[U-^{14}C]phenylalanine (group II), or L-[1-^{14}C]tyrosine (group III), or $NaH^{14}CO_3$ (group IV), or L-[U-^{14}C]tyrosine and L-[2,3-^{3}H]phenylalanine (group V) (all 1μCi/h). The solutions were infused for 6h while the animals were housed in Plexiglas chambers that permitted collection and analysis of expired breath. At the end of the 6h infusion, the rats were killed by decapitation, and mixed arterial and venous blood was collected from the neck into chilled heparinized containers. Plasma was separated from cellular constituents by centrifugation and stored at −30°C for later analysis.

An additional 12 animals (group VI) were infused with physiological saline ($n = 6$) or with 115–

120μmol of L-phenylalanine/h in physiological saline ($n = 6$) for only 4h, at which time they were killed by decapitation. Heparinized whole blood was separated into plasma and packed cells by centrifugation, and the plasma fraction kept at −30°C for analysis of amino acid concentrations.

Analytical methods

Total CO_2 production and specific radioactivity of expired breath were determined as previously described (Kawamura *et al.*, 1982). Briefly, rats were housed in Plexiglas chambers and air was drawn from the bottom of the chambers at a rate of 1.6l/min. The expired breath and room air were passed over $CaCl_2$ to trap moisture. Total CO_2 production was determined hourly by passing a timed collection (usually 15min) through weighing tubes containing $Ba(OH)_2$ (Ascarite; A. H. Thomas and Co., Philadelphia, PA, U.S.A.), by the technique of Wolfe & Burke (1977). $^{14}CO_2$ specific radioactivity was determined immediately by bubbling the dried expired breath into scintillation vials containing 200μmol of Hyamine hydroxide, ethanol, and phenolphthalein as an indicator. A commercial scintillant (Betaflur; National Diagnostics, Somerville, NJ, U.S.A.) was added directly to the scintillation vial and total ^{14}C radioactivity was determined in a Beckman LS-8000 liquid-scintillation spectrometer. All samples were counted for radioactivity until the standard error was less than 2.5%, and quenching was determined by using external standards.

In rats infused with L-[1-^{14}C]leucine (group I), plasma [^{14}C]leucine specific radioactivity was determined with an automated amino acid analyser (Moldawer *et al.*, 1980). Plasma L-[^{14}C]tyrosine specific radioactivity was determined enzymically by the method of Garlick & Marshall (1972) for rats infused wtih L-[U-^{14}C]tyrosine (group V). Plasma tyrosine specific radioactivities were not determined for rats infused with L-[1-^{14}C]tyrosine (group III), since the enzymic conversion of L-tyrosine into L-tyramine liberates $^{14}CO_2$ from the carboxy group (Garlick *et al.*, 1973). L-Phenylalanine specific radioactivities were determined fluorimetrically by using the technique of Garlick *et al.* (1980).

In rats infused with L-[U-^{14}C]phenylalanine, [^{14}C]phenylalanine and [^{14}C]tyrosine specific radioactivities were determined simultaneously. L-Tyrosine and L-phenylalanine specific radioactivities were measured after enzymic conversion of the amino acids into their β-amines, tyramine and β-phenethylamine respectively. The amines were extracted into organic solvents, re-extracted into weak mineral acids and analysed for specific radioactivity. The extraction of β-phenethylamine into chloroform/n-heptane (1:3, v/v) is specific and involves no contamination from L-tyramine (Garlick *et al.*, 1980), but dichloroethane extraction of tyramine results in appreciable extraction of β-phenethylamine. Therefore the specific radioactivity of L-tyrosine during infusion of L-[U-^{14}C]phenylalanine was determined indirectly by measuring the β-phenethylamine specific radioactivity in the chloroform/n-heptane extraction and the total ^{14}C-radioactivity, tyramine and β-phenethylamine concentrations in the dichloroethane extraction. The specific radioactivity of β-phenethylamine obtained from the chloroform/n-heptane extraction was then multiplied by the total β-phenethylamine concentration in the dichloroethane extraction, and the corresponding radioactivity (d.p.m.) was subtracted to leave total tyramine concentration and remaining ^{14}C radioactivity. A similar procedure was used for estimating [^{3}H]tyrosine specific radioactivity during simultaneous infusion of L-[2,3-^{3}H]phenylalanine and L-[U-^{14}C]tyrosine. The specific radioactivities of L-tyramine and β-phenethylamine were assumed to be identical with those of their corresponding amino acids.

Amino acid concentrations in plasma samples from rats in groups I and VI were determined on sulphosalicylic acid supernatants with an automated amino acid analyser (D-400; Dionex, Sunnyvale, CA, U.S.A.).

Calculations

Plasma amino acid appearance was determined from the dilution of radiolabelled amino acid in the plasma compartment:

$$Q = I/Sp_{max.}$$

where Q is the appearance of amino acid, in μmol/h, I is the infusion rate of radioisotope, in d.p.m./μmol, and $Sp_{max.}$ is the specific radioactivity of amino acid in the plasma compartment, in d.p.m./μmol. Rates of oxidation of ^{14}C-labelled amino acid were obtained from the appearance of $^{14}CO_2$ in the expired breath when isotopic steady states were achieved, and from the plasma specific radioactivity, by using the equation:

$$O = E/Sp_{max.}$$

where O is the oxidation rate, in μmol/h, and E is the appearance of $^{14}CO_2$ in the expired breath, in d.p.m./h. Tyrosine oxidation rates were measured in rats infused with L-[U-^{14}C]tyrosine (group V) and rats infused with L-[U-^{14}C]phenylalanine (group II); the specific radioactivity of plasma [^{14}C]tyrosine was used in both studies as being representative of the precursor pool for whole-body tyrosine oxidation.

The percentage of metabolically generated carbonate that did not appear as CO_2 but was either retained in the body or excreted as other products

was estimated from the 6h infusion of $NaH^{14}CO_3$ (group IV).

The percentage of plasma phenylalanine appearance converted into plasma tyrosine was obtained from rats infused simultaneously with L-[U-^{14}C]-tyrosine and L-[2,3-^{3}H]phenylalanine (group V) by the equation:

$$\% \text{ converted} = \frac{I([^{14}C]Tyr)}{Sp_{max.}([^{14}C]Tyr)} \times \frac{Sp_{max.}([^3H]Tyr)}{I([^3H]Phe)} \times 100$$

where $I([^{14}C]Tyr)$ is the infusion rate of L-[U-^{14}C]tyrosine, in d.p.m./h, $Sp_{max.}([^{14}C]Tyr)$ is the [^{14}C]tyrosine specific radioactivity, in d.p.m./μmol, $Sp_{max.}([^3H]Tyr)$ is the [^{3}H]tyrosine specific radioactivity, in d.p.m./μmol, and $I([^3H]Phe)$ is the infusion rate of L-[2,3-^{3}H]phenylalanine, in d.p.m./h. The percentage of phenylalanine flux converted into tyrosine was then multiplied by the plasma phenylalanine flux (^{3}H) to obtain total conversion rates, in μmol/h.

Data were evaluated by Student's *t* test. Differences were considered significant when the level of confidence exceeded 95%.

Results

The appearance of $^{14}CO_2$ in expired breath from rats infused with different ^{14}C-labelled amino acids or $NaH^{14}CO_3$ is presented in Fig. 1. In rats infused with $NaH^{14}CO_3$ the percentage of infused radioisotope appearing in the expired breath was 76 ± 5% (mean ± S.E.M.; group IV) and did not differ between animals infused with only physiological saline or the L-phenylalanine solution. As a result the data have been combined, and oxidation rates for individual ^{14}C-labelled amino acids have been calculated by assuming that 24% of metabolically generated CO_2 had been retained in the body.

The administration of 115–120μmol of L-phenylalanine/h increased plasma concentrations of phenylalanine and tyrosine and produced a slight decrease in plasma leucine (Table 1). Leucine appearance and oxidation were only minimally changed by the intravenous infusion of 115–120μmol of L-phenylalanine/h (Table 2). In post-absorptive rats, 18.3μmol/h, or 26.6%, of plasma appearance was oxidized entirely to CO_2 and the L-phenylalanine infusion decreased it insignificantly to 14.0μmol/h, representing 23.8% of plasma appearance.

In post-absorptive rats infused with L-[U-^{14}C]-tyrosine and L-[U-^{14}C]phenylalanine (group II), rates of appearance of plasma tyrosine and phenylalanine were remarkably similar (Table 3). Of the 42.1μmol of tyrosine/h appearing in the plasma, 9.1μmol/h, or 21.6%, of plasma tyrosine appearance was due to the conversion of phenylalanine.

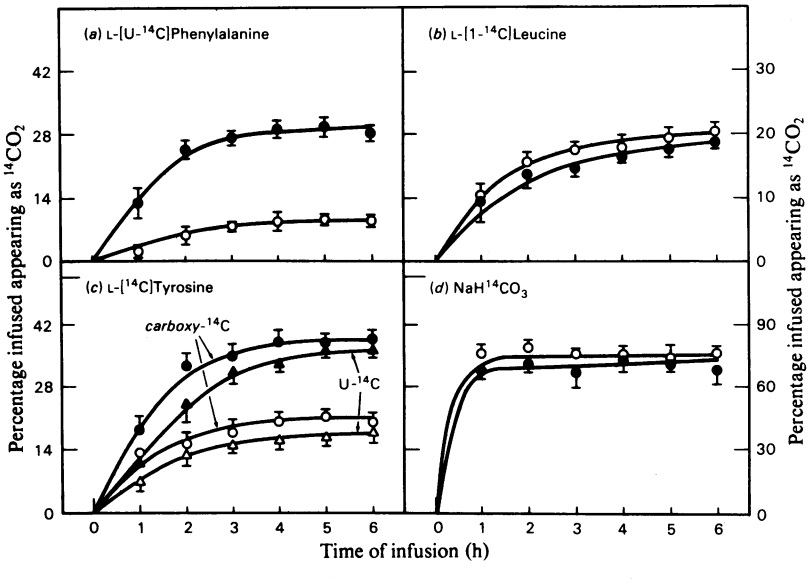

Fig. 1. *Attainment of isotopic steady states in expired CO_2*

Rats were infused with (*a*) L-[U-^{14}C]phenylalanine, (*b*) L-[1-^{14}C]leucine, (*c*) L-[^{14}C]tyrosine (●, ○, *carboxy*-^{14}C; ▲, △, U-^{14}C) or (*d*) $NaH^{14}CO_3$ for 6h through an indwelling catheter placed in the lateral tail vein. Half of the animals in each group received only physiological saline, and the remainder were infused with 115–120μmol of L-phenylalanine/h in physiological saline. Within 4–6h, isotopic steady states were achieved in the expired breath of all animals. ○, △, Post-absorptive rats: ●, ▲, phenylalanine-loaded rats.

Table 1. *Selected plasma amino acid concentrations in post-absorptive and phenylalanine-loaded rats*
After overnight starvation, indwelling catheters were placed in the lateral tail vein of 90–120g rats and the animals were infused with either physiological saline or 115–120μmol of L-phenylalanine/h for 4–6h. Rats were killed by decapitation and plasma was analysed for free amino acid concentrations as described in the Experimental section. Differences in amino acid concentrations between rats infused for 4h (group VI) or 6h (group I) were not significant. However, rats that received the phenylalanine infusion had significantly higher plasma concentrations of phenylalanine and tyrosine than did animals receiving only physiological saline (**$P < 0.01$). Values represent the means ± S.E.M. for six aminals.

	Post-absorptive		Phenylalanine-loaded	
	4h (6)	6h (6)	4h (6)	6h (6)
L-Leucine (nmol/ml)	221 ± 16	235 ± 19	188 ± 24	174 ± 20
L-Phenylalanine (nmol/ml)	75 ± 8	83 ± 3	610 ± 99**	664 ± 136**
L-Tyrosine (nmol/ml)	60 ± 6	53 ± 5	201 ± 28**	214 ± 28**

Table 2. *Plasma leucine kinetics (group I)*
Post-absorptive rats were infused with either physiological saline or 115–120μmol of L-phenylalanine/h, to which 1μCi of L-[1-^{14}C]leucine/h was added. Values represent the means ± S.E.M. for six animals in each group, and the differences between groups were not statistically significant.

	Post-absorptive	Phenylalanine-loaded
Plasma leucine appearance (μmol/h)	68.9 ± 3.0	58.9 ± 4.0
Leucine oxidation (μmol/h)	18.3 ± 2.1	14.0 ± 1.2
Leucine incorporation into whole-body protein (μmol/h)	50.6 ± 1.8	44.9 ± 5.1
Percentage of leucine appearance oxidized	26.6 ± 2.1	23.8 ± 1.7

Table 3. *Phenylalanine and tyrosine kinetics in post-absorptive and phenylalanine-loaded rats*
Rats were infused with physiological saline or 115–120μmol of L-phenylalanine/h, and added to each solution were either L-[U-^{14}C]phenylalanine (Group II) or L-[2,3-^{3}H]phenylalanine and L-[U-^{14}C]tyrosine (Group V). All of the differences in plasma tyrosine and phenylalanine flux and oxidation, as well as the conversion of phenylalanine into tyrosine, were statistically significant (*$P < 0.05$) between post-absorptive and phenylalanine-loaded rats. In addition, estimates of tyrosine oxidation based on the continuous infusion of [^{14}C]phenylalanine and [^{14}C]tyrosine were also significantly different. Values represent means ± S.E.M. for the numbers of animals shown in parentheses.

	Post-absorptive	Phenylalanine-loaded
Plasma phenylalanine appearance (μmol/h)	43.7 ± 9.1 (7)	166 ± 17.6 (7)
Plasma tyrosine appearance (μmol/h)	42.1 ± 4.6 (7)	79.3 ± 9.0 (6)
Conversion of plasma phenylalanine into plasma tyrosine (μmol/h)	9.1 ± 1.3 (7)	56.8 ± 8.4 (6)
Phenylalanine oxidation (μmol/h)	5.3 ± 1.1 (6)	62.1 ± 9.4 (6)
Tyrosine oxidation (μmol/h)		
L-[U-^{14}C]Tyrosine	10.3 ± 0.8 (7)	38.1 ± 7.7 (6)
L-[U-^{14}C]Phenylalanine	27.3 ± 5.1 (6)	89.2 ± 12.4 (6)

From the L-[U-^{14}C]tyrosine infusion, 24.4% of plasma tyrosine appearance, or 10.3μmol of plasma tyrosine/h, was oxidized.

In post-absorptive rats infused with L-[U-^{14}C]-phenylalanine (group II), phenylalanine oxidation rates were 5.3μmol/h, representing 12.1% of its plasma appearance (Table 3). However, apparent tyrosine oxidation rates in these animals, based on the plasma [^{14}C]tyrosine specific radioactivity, were 27.3 ± 5.1μmol/h, or 2.65 times that observed in similar post-absorptive rats infused with L-[U-^{14}C]-tyrosine.

The infusion of 115–120μmol of L-phenylalanine/h significantly increased plasma phenylalanine and tyrosine appearance and oxidation. The percentages of plasma tyrosine and phenylalanine appearance that were oxidized increased to 48.0% and 37.3% respectively ($P < 0.001$). In rats infused with 115–120μmol of phenylalanine/h, conversion of plasma phenylalanine into plasma tyrosine was 56.8μmol/h, which represented 71.6% of total plasma tyrosine appearance. When plasma phenylalanine and tyrosine oxidation rates were derived from rats infused with 115–120μmol of L-phenylalanine/h and L-[U-^{14}C]phenylalanine (group II), oxidation rates were 62.1 and 89.2μmol/h respectively. However, when plasma tyrosine oxidation rates were obtained in rats infused with 115–120μmol of L-phenylalanine and L-[U-^{14}C]tyro-

Table 4. *Differences in the oxidation of uniformly and carboxy-labelled tyrosine in post-absorptive amd phenylalanine-loaded rats*

Post-absorptive rats were infused for 6h with either physiological saline or 115–120μmol of L-phenylalanine/h to which L-[1-^{14}C]- or L-[U-^{14}C]-tyrosine (1μCi/h) was added. The difference between L-[U-^{14}C]tyrosine and L-[1-^{14}C]tyrosine oxidation represented the quantity of plasma tyrosine initially deaminated and decarboxylated but not degraded to CO_2 and either retained in the body or excreted as other products. Values represent the means ± S.E.M. for five to seven animals.

	Post-absorptive		Phenylalanine-loaded	
	L-[U-^{14}C]Tyrosine ($n = 7$)	L-[1-^{14}C]Tyrosine ($n = 5$)	L-[U-^{14}C]Tyrosine ($n = 6$)	L-[1-^{14}C]Tyrosine ($n = 5$)
Plasma tyrosine appearance (μmol/h)	42.1 ± 4.6	—	79.3 ± 9.0	—
Percentage of flux oxidized	24.4 ± 2.2	27.8 ± 2.2	48.0 ± 6.2	50.5 ± 7.8
Oxidation (μmol/h)	10.3	11.7	38.1	40.1
Partially degraded and retained in the body or excreted (μmol/h)	1.4		2.0	

sine/h, tyrosine oxidation rates were only 38.1μmol/h.

The percentages of L-[1-^{14}C]tyrosine (group III) and L-[U-^{14}C]tyrosine (group V) appearing in the expired breath are summarized in Table 4. Complete oxidation of L-[1-^{14}C]tyrosine was greater than that of L-[U-^{14}C]tyrosine, but the differences were not significant and represented only a 2.4% and 1.9% difference in the quantity of infused isotope retained in post-absorptive and phenylalanine-loaded animals, respectively.

Discussion

This study more fully characterizes the interrelationship between phenylalanine and tyrosine metabolism *in vivo*. In the post-absorptive rat, approx. 22% of plasma tyrosine was derived from phenylalanine. Similar findings are reported in man (16%; Clark & Bier, 1982). Results are also consistent with the 0.11–0.15μmol of tyrosine/min (7–9μmol/h) for phenylalanine hydroxylation reported in the 100–150g rat (Milstein & Kaufman, 1975).

These findings also emphasize the importance of dietary phenylalanine intake in altering plasma tyrosine kinetics. In rats intravenously infused with 115–120μmol of L-phenylalanine/h, representing three times the rate of appearance in the post-absorptive state, plasma tyrosine flux increased by 88% and 56.8μmol/h, or 72% of total tyrosine appearance was derived from phenylalanine.

The increased proportion of phenylalanine appearance converted into plasma tyrosine, as well as the greater percentages of uniformly and carboxy-labelled tyrosine oxidized in response to an intravenous phenylalanine load, suggests that the catabolism of excess dietary phenylalanine is mediated by increases in both the hydroxylation of phenylalanine as well as the subsequent oxidative decarboxylation of tyrosine.

Of interest, however, is the profound difference between rates of tyrosine oxidation derived from the continuous infusion of L-[U-^{14}C]phenylalanine and L-[U-^{14}C]tyrosine. In post-absorptive rats, estimates of tyrosine oxidation were either 10.3 or 27.3μmol/h, depending on whether animals were infused with [^{14}C]tyrosine or [^{14}C]phenylalanine respectively. A primary assumption of all kinetic studies with labelled amino acids is that the total free amino acid pool of the body is well mixed and represented by enrichments in the plasma compartment. Regardless of whether rats were infused with [^{14}C]phenylalanine or [^{14}C]tyrosine, simultaneous measurements of expired $^{14}CO_2$ and plasma [^{14}C]tyrosine specific radioactivity should give equivalent estimates of whole-body tyrosine oxidation. In practice, however, since the tracer is generally infused into the plasma pool and the site of oxidation is intracellular, where tracer enrichments are lower, oxidation rates derived from plasma specific radioactivities are always somewhat underestimated. This underestimate is usually considered to be minimal (20%) and relatively constant under various nutritional and metabolic circumstances.

The present work, however, demonstrates that the latter assumptions are probably not acceptable for the phenylalanine–tyrosine system. The considerable differences in tyrosine oxidation based on continuous infusions of either L-[U-^{14}C]tyrosine or L-[U-^{14}C]phenylalanine can be explained in part by compartmentalization of tyrosine between the plasma pool and the site of phenylalanine hydroxylation.

Compartmentalization of tyrosine between plasma and 'acid-soluble' fractions of individual

tissues has been extensively reported (Garlick *et al.*, 1973, 1975; Sakamoto *et al.*, 1983). In the post-absorptive rat, approx. 50% of hepatic free tyrosine is not derived from the plasma compartment, but rather from intracellular sources, as a result of either protein degradation or synthesis *de novo*. Because of incomplete mixing between extracellular and intracellular pools, the site of isotope administration will have considerable impact on whole-body estimates of tyrosine oxidation.

The difference between estimates of tyrosine oxidation with [^{14}C]phenylalanine and [^{14}C]tyrosine is consistent with the explanation that some quantity of tyrosine generated from phenylalanine hydroxylation is further degraded within the hepatocyte without first equilibrating with the plasma. Under these conditions, phenylalanine that is completely oxidized without the newly synthesized tyrosine appearing in the plasma pool is measured as [^{14}C]phenylalanine oxidation (group II), but not as [^{14}C]tyrosine oxidation (from a [^{14}C]tyrosine infusion) or as appearance of [^{3}H]tyrosine during an infusion of [^{3}H]phenylalanine (group V).

The intravenous infusion of radiolabelled phenylalanine simulates hepatic intracellular administration of radiolabelled tyrosine, since the hydroxylation of phenylalanine occurs in the cytosolic matrix of the hepatocyte. The subsequent specific radioactivity of tyrosine in the plasma compartment would reflect mixing of both intracellular and plasma compartments. Therefore plasma tyrosine specific radioactivities obtained during the course of a radiolabelled-phenylalanine infusion would be less than the specific radioactivity of the true precursor pool for tyrosine oxidation. This is because the plasma tyrosine specific radioactivity would reflect additional dilution of the tracer by unlabelled tyrosine released into the plasma compartment from protein degradation, which is occurring in non-hepatic tissues.

It can be concluded that phenylalanine metabolism plays a significant role in the plasma appearance of tyrosine and that the hydroxylation of phenylalanine is sensitive to dietary phenylalanine intake. It is clear from these studies, however, that oxidation of tyrosine determined from the dilution of L-[U-^{14}C]tyrosine in the plasma compartment is an underestimate of true rates, but not because uniformly labelled metabolites of tyrosine degradation are retained. Rather, tyrosine oxidation is underestimated because of compartmentalization of the amino acid between the plasma and intracellular pools and/or a preferential oxidation of tyrosine generated from the hydroxylation of phenylalanine.

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