Communications to the Editor

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THE FORMATION OF m-TYROSINE AND o-TYROSINE IN RATS

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Intraperitoneal administration of L-phenylalanine in rats gave rise to three hydroxylated products which were identified by high performance liquid chromatography as p-tyrosine, m-tyrosine and o-tyrosine.

KEYWORDS —— phenylalanine ; p-tyrosine ; m-tyrosine ; rat ; o-tyrosine ; intraperitoneal administration ; fluorescence high performance liquid chromatography

It is well known that phenylalanine is metabolized largely by conversion to p-tyrosine (tyrosine) in mammals.[1] On the other hand, many authors have reported the presence of m-tyramine[2] and o-tyramine[3] as constituents of human urine, and it has been demonstrated that all these compounds can be derived from m-tyrosine (m-hydroxyphenylalanine) and o-tyrosine (o-hydroxyphenylalanine) in the human body.[4] These findings suggest that m-tyrosine and o-tyrosine may be formed in mammals. The first indication that the formation of m-tyrosine may be associated with the hydroxylation of phenylalanine came from the study of Tong et al.,[5] in which phenylalanine was treated with bovine adrenal medulla homogenate in the presence of both a pteridine co-factor and a DOPA decarboxylase inhibitor. Recently, we also reported the formation of m-tyrosine and o-tyrosine from phenylalanine *in vitro* by a rat brain preparation in the presence of a pteridine co-factor,[6] and the existence of m-tyrosine in human plasma.[7] However, the formation of m-tyrosine and o-tyrosine *in vivo* was not yet found. The present communication describes the formation of m-tyrosine and o-tyrosine following intraperitoneal administration of phenylalanine in rats.

Male Wistar rats weighing 200 to 250 g were used after fasting for 18 h. Phenylalanine dissolved in 0.9% NaCl solution was administered intraperitoneal (i.p.) to rats in doses of 400 mg/kg. Blood was collected from the abdominal aorta and centrifuged at 3000 rpm for 5 min to obtain the serum. A 2 ml sample of the serum was deproteinized by the addition of 0.5 ml of 1.0 M trichloroacetic acid. After centrifuging down the precipitate, 50 µl of the supernatant was subjected to a fluorescence high performance liquid chromatography (HPLC). A typical chromatographic pattern is shown in Fig. 1. These chromatographic

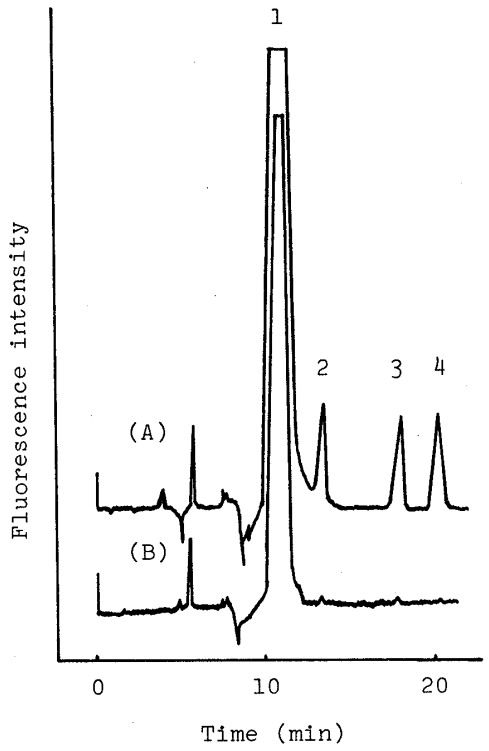

Fig. 1 Chromatograms of HPLC of the Rat Serum

(A) Injection sample: Serum sample obtained from the rat 30 min after intraperitoneal administration of phenylalanine.

(B) Injection sample: Serum sample obtained from the intact rat.

A 50 µl portion was introduced to HPLC under the following conditions: column, Cosmosil C_{18} (15 cm × 4.6 mm i.d.); mobile phase, 0.1% acetic acid containing 0.1% sodium chloride; flow rate, 1 ml/min; detection, excitation at 275 nm and emission at 305 nm.

Peaks : 1 = p-tyrosine; 2 = m-tyrosine; 3 = o-tyrosine; 4 = phenylalanine.

analyses indicated that the concentration of m-tyrosine and o-tyrosine as well as p-tyrosine in the serum was significantly increased by the i.p. administration of phenylalanine. Under these chromatographic conditions, the retention time of 3,4-dihydroxyphenylalanine, catecholamines, phenylpyruvic acid and 3-methoxy-tyrosine, clearly differed from those of p-, m-, and o-tyrosine.

The serum concentration of p-tyrosine, m-tyrosine and o-tyrosine after i.p. administration of phenylalanine in rats is shown in Table I. Before

Table I. The Serum Concentration of Hydroxyphenylalanines after Intraperitoneal Administration of Phenylalanine in Rats

Time[1] (min)	Tyrosine formed		
	p- (µg/ml)	m- (ng/ml)	o- (ng/ml)
0	12.5 ± 1.6	3.3 ± 1.9	3.6 ± 1.8
30	55.0 ± 11.4	35.3 ± 9.3	40.2 ± 7.7
60	64.3 ± 9.1	38.1 ± 10.1	41.4 ± 12.8
90	72.6 ± 9.6	37.3 ± 11.4	39.0 ± 10.6
120	64.9 ± 9.4	20.0 ± 7.8	24.0 ± 9.2
150	48.8 ± 5.1	11.3 ± 4.3	12.1 ± 4.9
180	30.4 ± 4.0	5.0 ± 1.1	5.3 ± 1.0

Values are means ± S.D. of three experiments.

1) Time after the administration of phenylalanine.

administration, the concentrations of the compounds were : p-tyrosine, 12.5 ± 1.6 μg/ml ; m-tyrosine, 3.3 ± 1.9 ng/ml ; o-tyrosine, 3.6 ± 1.8 ng/ml. The concentration of the tyrosine isomers in the serum was increased with time by the administration of phenylalanine and reached the maximum at around 60 to 90 min after the administration and thereafter declined to their normal levels. These results indicate that a part of the phenylalanine administered in rat is hydroxylated to m-tyrosine and o-tyrosine.

The present study suggests that m-tyrosine and o-tyrosine, besides p-tyrosine are also formed from phenylalanine *in vivo*. More detailed investigations of the formation of m-tyrosine and o-tyrosine in other methods of administration of phenylalanine and studies of the mechanism of hydroxylation to m-tyrosine and o-tyrosine from phenylalanine *in vivo* are now in progress in our laboratory.

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