TRYPTOPHAN DECARBOXYLATION: A QUANTITATIVELY SIGNIFICANT ROUTE OF TRYPTOPHAN METABOLISM

Jean-Claude DAVID

Institut de Biologie Moleculaire, Faculté des Sciences, Place Jussieu, Paris Ve, France

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1. Introduction

We have previously presented findings which indicated that decarboxylation of the aromatic amino acids becomes a major pathway for their metabolism when their tissue levels are raised [1,2]. This conclusion was based in part on the finding that the plasma levels attained following oral administration of large amounts of one of these amino acids could be further elevated (over two-fold) by administrating an aromatic-L-amino acid decarboxylase inhibitor [1]. Subsequently more direct and quantitative studies were reported on the contribution of decarboxylation to the metabolism of tyrosine [2]. It was shown that in mice the proportion of tyrosine metabolized via decarboxylation increased up to 42% of the dietary intake with increasing intake of the amino acid. At higher levels of intake decarboxylation was quantitatively more significant than transamination. The present experiments were designed to obtain a quantitative measure of the role of decarboxylation in the metabolism of tryptophan.

2. Materials and methods

Male MF_1 mice (25–30 g), obtained from Marland Farms, Wayne, New Jersey, were used in these studies. To minimized the possible effects of gastrointestinal flora on the metabolism of orally administered tryptophan, the mice were deprived of food and 2 g/l of neomycin sulfate was placed in the drinking water 24 hr prior to the initiation of all experiments [3]. In addition the mice were given 2 mg/kg of sulfasuxidine by mouth 24 hr and immediately prior to the initiation of all experiments.

RO4-4602 [N_1-D-seryl)-N_2-(2,3,4 dihydroxybenzylhydrozine)] was a gift of Hoffmann-LaRoche, Ltd. Nutley, New Jersey. Carboxyl labeled L-tryptophan (10 Ci/mol) were obtained from New England Nuclear. Results were corrected for quenching by means of an infernal standard and are expressed in dpm.

Mice were given carboxyl-^{14}C-labeled L-tryptophan orally in the presence of varying amounts of non-radioactive tryptophan and immediatelv placed in separate 500 ml Erlenmeyer flasks and the expired air was continuously passed through two consecutive flasks each containing 100 ml of 10 N NaOH to trap the CO_2. Six hr following the administration of the carboxyl labeled tryptophan, the radioactivity in each trap was determined by liquid scintillation counting.

3. Results

The efficiency and the capacity of the collecting system for CO_2, was determined as follows. Five control mice were placed in the collecting system and the expired air was passed through the sodium hydroxide solutions. After six hr, the mice were removed from the Erlenmeyer flask and a known amount of $Na_2[^{14}C]O_3$ was added in their place and the system resealed. Five ml of concentrated sulfuric acid was then introduced into the flask and the $^{14}CO_2$ was collected for 30 min. It was found that 97% of the radioactivity originally added to the flask could be trapped in the sodium hydroxide solution. These results indicated that the system used was sufficient to collect all the CO_2 expired by the mice during the six hr period.

The amount of radioactive CO_2 collected from the experimental mice, increased with increasing amounts

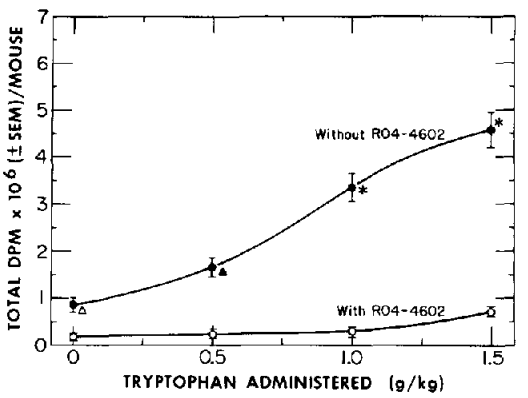

Fig.1. Expired $^{14}CO_2$ following administration of carboxyl labeled tryptophan as a function of the tryptophan intake. Mice were given the decarbozylase inhibitor, Ro 4–4602, (50 mg/kg/i.p.), the indicated amounts of non-radioactive tryptophan (p.o.), and 10.2 × 10^6 dpm of carboxyl-labeled tryptophan. Expired $^{14}CO_2$ was collected in sodium hydroxide as described in the text. Five mice were used for the determination of each point. Results are expressed as dpm of expired CO_2 per mouse per 6 hr ± S.E.M. (△) Significantly different from Ro 4-4602 treated animals (p <0.01). (▲) Significantly different from Ro 4-4602 treated animals (p <0.005). (*) Significantly different from Ro 4–4602 treated animals (p <0.001).

of administered tryptophan (see fig.1). When only tracer amounts of carboxyl labeled tryptophan was administered approximately 8% of the radioactivity was collected as expired CO_2. As the dose was progressively increased from 0.5 to 1.0 to 1.5 g/kg, this increased to 16.3, 30.5 and 42% respectively. As is also shown in Fig.1, when the decarboxylase inhibitor, Ro 4–4602, was given the appearance of $^{14}CO_2$ in the expired air was markedly diminished. These findings are consistent with the interpretation that the $^{14}CO_2$ originates through the decarboxylation of tryptophan.

Another group of mice were given 3-[^{14}C] tryptophan so that the newly synthesized tryptamine formed through decarboxylation would retain the ^{14}C-radioactivity. These mice were pretreated with the monoamine oxidase inhibitor pargyline (50 mg/kg i.p.) 24 hr and 1 hr prior to the initiation of the experiment. This compound was given to prevent further metabolism of tryptamine by oxidative deaminations. Six hours following the administration of 3-[^{14}C] tryptophan, the animals were killed and the entire carcass, minus the gastro-intestinal tract, plus any voided urine was homogenized in 3 vol of water and treated as previously described [2]. Tryptamine was isolated from the trichloroacetic-acid-soluble fraction by several chromatographic steps on IRC 50 resin. The trichloroacetic acid extract was passed through a 1 × 5 cm column previously adjusted to pH 6.5 and the effluent discarded. The column was washed with 5 ml of water followed by 5 ml of 0.5 M phosphate buffer pH 6.5 and the washings and eluates were discarded. The basic fraction was then eluted with 5 ml of 4 N ammonia. Authentic tryptamine was eluted in the final basic fraction with a recovery varying between 85 and 90%.

Shown in table 1 are the amounts of radioactivity found in the basic fraction six hr following the administration of 3-[^{14}C] tryptophan. As the oral dose of tryptophan was increased from the tracer amount to 0.5, 1.0 and 1.5 g/kg, the radioactivity found in the tryptamine-associated fraction increased from 10.8%, to 16.8%, 30.5% and 43% of the administered tryptophan radioactivity.

These data are in excellent agreement with those found on evolved $^{14}CO_2$ (fig.1). Thus, administration of the decarboxylase inhibitor essentially prevented

Table 1
Extent of decarboxylation as a function of increasing tryptophan intake

Dose of tryptophan (g/kg)	dpm × 10^6/mouse ± S.E.M. NaCl 0.9%	Ro 4–4602
Tracer[a]	1.088 ± 0.08	0.109 ± 0.012
0.5	1.504 ± 0.10	0.127 ± 0.008
1.0	2.720 ± 0.18	0.126 ± 0.005
1.5	3.835 ± 0.12	0.186 ± 0.004

Mice were given orally 10.5 × 10^6 dpm of 3-[^{14}C] tryptophan in 0.3 ml of distilled water in the presence or absence of three different amounts of non-radioactive tryptophan (0.5, 1.0, 1.5 g/kg) and divided in two groups. At the same time the first group received NaCl (0.9% i.p.) and the second group the decarboxylase inhibitor, Ro 4–4602 (50 mg/kg i.p.). The animals were killed six hr later and the radioactivity in the basic fraction determined as described in the test. Four animals were used for the determination of each point.

[a] The radioactive tracer amount of tryptophan was equivalent to $5.5 \cdot 10^{-5}$ g/kg.

the appearance of radioactivity in the basic fraction, a result consistent with the formation of tryptamine via the decarboxylation pathway.

Some of the radioactivity found as CO_2 or eluted in the basic fraction from the IRC column could have arisen by decarboxylation of 5-hydroxtryptophan formed from tryptophan. To investigate this tryptamine and 5-hydroxytryptamine were separated by paper chromatography (Whatman 3 MM butanol–acetic acid–water 6:1.5:2.5 v/v/v). It was found that at a dose of 1.5 g/kg of tryptophan, 90% of the radioactivity in the basic fraction migrated with tryptamine, and 10% with 5-hydroxytryptamine. At a dose of 1 g/kg, only 5% were associated with 5-hydroxytryptamine. At the tracer and 0.5 g/kg doses, all the radioactivity was associated with tryptamine.

4. Discussion

It has been know for nearly 40 years that tryptophan can be converted to kynurenine through the tryptophan pyrrolase (EC 1.13.11.11) pathway [4]. This pathway has been considered to be quantitatively the most important metabolic route for this amino acid [5–7]. Recently, Hayaishi et al. [8] have described an indoleamine 2,3-dioxygenase which utilizes tryptophan as a substrate and is believed to be of some importance in the intestinal wall and brain [8,9]. The formation of tryptamine has been considered to be a minor route of tryptophan metabolism. We have shown in this report that the decarboxylation pathway for tryptophan is by no means a minor route either during fasting or during high tryptophan intake. These findings with tryptophan are similar to those we have previously reported for tyrosine [1,2]. Since phenylalanine is an excellent substrate of aromatic-L-amino acid decarboxylase it is likely that decarboxylation is also an important route for its metabolism.

The amines corresponding to the aromatic amino acids possess appreciable activity on nerve and smooth muscle. However, most of the decarboxylation takes place in liver and kidney which are rich in monoamine oxidase. Apparently this enzyme is capable of inactivating the bulk of the generated amines. Nevertheless, one wonders whether or not these large amounts of tyramine, tryptamine and phenylethylamine which are formed play a physiological role. Even if that were so, one must consider the possibility of amine involvement in some pathologic states.

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