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DECARBOXYLATION OF 1,2,3,4-TETRAHYDRO-β-CARBOLINE-1-CARBOXYLIC ACIDS IN BRAIN HOMOGENATE AND CATALYSIS BY PYRIDOXAL-5′-PHOSPHATE

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Abstract—[Carboxyl-^{14}C] labelled 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (I) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (II) were synthesized and their decarboxylation was studied in mouse brain homogenate and buffer. The decarboxylation rates of (I) and (II) in the homogenate were about 6-fold and 4-fold, respectively, as compared with the rates in phosphate buffer. The increase could not be prevented by preheating the homogenate, but was partially abolished by addition of 1 mM EDTA. The decarboxylation was increased dose-dependently when pyridoxal-5′-phosphate was included in the buffer, 400 μM being sufficient to exceed the rate in homogenate for both (I) and (II). Mass spectrometric examination of the decarboxylation products indicated that both (I) and (II) were degraded mainly to corresponding 1,2,3,4-tetrahydro-β-carbolines, but some 3,4-dihydro analogues also were detectable. In conclusion, the results outline a way through which these pharmacologically active β-carbolines are readily formed under conditions that may be regarded as physiological.

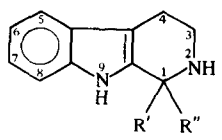
Several 1,2,3,4-tetrahydro-β-carbolines (THBC)‡ (Fig. 1) have been reported to exist in trace amounts in human and animal tissues [1–5]. Some of the findings may be uncertain due to methodological difficulties [6, 7], but increasing evidence is accumulating for the occurrence of at least THBC, 1-methyl-THBC and 6-methoxy-THBC [8–12]. The THBCs possess certain pharmacological properties like inhibition of monoamine oxidase-A (EC 1.4.3.4.) [13, 14] and 5-hydroxytryptamine uptake [15], and binding to tryptamine [16], opiate [17], 5-hydroxytryptamine [18], imipramine [19] and benzodiazepine [20] binding sites. They have also hallucinogenic [20], tremorgenic [21] and convulsive [22] effects on humans and other mammals.

The endogenous THBCs are supposed to be derived from tryptamine either via reactions with biogenic aldehydes or keto acids (Fig. 2). In the case of formaldehyde and acetaldehyde it has been proposed that the THBCs are formed spontaneously through the Pictet-Spengler reaction [23, 24]. However, this reaction between the aldehydes and tryptamine or its derivatives appears to be slow and gives poor yields at 37° and neutral pH [25]. On the other hand, reactions between tryptamine structures and glyoxylic or pyruvic acid, yielding the THBC-1-carboxylic acids proceed much faster under corresponding conditions [25, 26], and it has been recently reported that carboxylic derivatives are transformed to corresponding THBCs in rat tissues *in vivo* [27]. To study the requirements and the rate of decarboxylation *in vitro* we synthesized both unlabelled and [carboxyl-^{14}C] labelled THBC-1-carboxylic acid (I) and 1-methyl-THBC-1-carboxylic acid (II), and examined the reactions in mouse brain homogenate and buffer systems. Particular interest was focused on the probable role of pyridoxal-5′-phosphate (PLP), the principal co-factor in biological decarboxylations (cf. [28, 29]).

‡ Abbreviations used: I, 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid; II, 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid; PLP, pyridoxal-5′-phosphate; THBC, 1,2,3,4-tetrahydro-β-carboline.

MATERIALS AND METHODS

Synthesis of substrates and reference molecules. The compounds (I) and (II) investigated were synthesized as described in ref. 30 from tryptamine hydrochloride and glyoxylic or pyruvic acid, respectively, which were purchased from Fluka AG (Buchs SG, Switzerland). For the ^{14}C-labelled compounds



R′	R″	
H	COOH	THBC-1-carboxylic acid (I)
CH_3	COOH	1-Me-THBC-1-carboxylic acid (II)
H	H	THBC
CH_3	H	1-Me-THBC

Fig. 1. Structures of the 1,2,3,4-tetrahydro-β-carbolines studied.

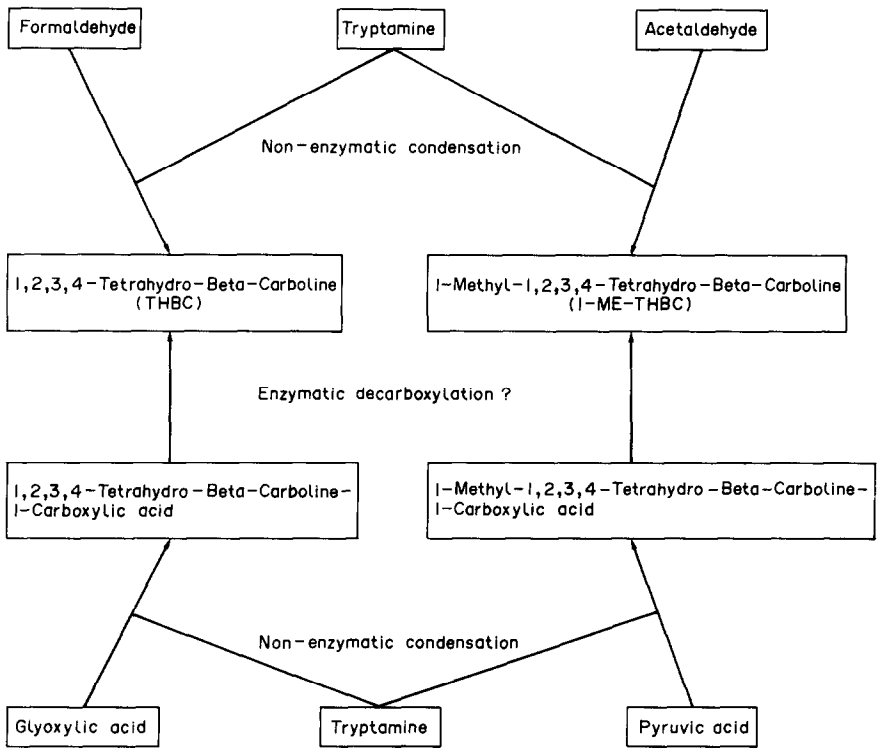


Fig. 2. Scheme of the routes suggested for the formation of endogenous β-carbolines.

[1-^{14}C]glyoxylic acid (sp. act. 7.33 mCi/mmol) and [1-^{14}C]pyruvic acid (sp. act. 23 mCi/mmol) from Amersham (Bucks, U.K.) were used accordingly. The molecules used as reference materials in mass spectrometry were synthesized and analyzed by MS, HNMR, ^{13}CNMR and IR, and their purity was verified by TLC as outlined in refs. 30–32.

Decarboxylation experiments. Liberation of CO_2 from the radiolabelled (I) and (II) was investigated in 100 mM potassium phosphate buffer (pH 7.4 at 20°). Mouse brains were homogenized in 5-fold amount (w/v) of the buffer using a Potter-Elvehjem homogenizer. The concentrations of (I) and (II) were 250 μM at respective specific activities of 1.7 and 1.0 mCi/mmol in a final reaction volume of 200 μl. Other constituents or treatments were applied as specified under Results. The assays were carried out in 10 ml conical bottomed polyethylene tubes, which were equipped with polypropylene center wells attached to rubber stoppers (Kontes Glass Co., Vineland, NJ). After the reaction period of 1 hr 1 ml of 50% (w/v) trichloroacetic acid was injected into the tubes to stop possible enzyme action and to volatilize any dissolved CO_2 from the reaction mixture. Then the tubes were kept for 30 min at room temperature to ascertain the absorption of CO_2 into the 300 μl of ethylene glycol-ethanolamine (1:1 v/v) in the center wells. For measurement of radioactivity the wells were inserted in liquid scintillation counting vials containing 4 ml ACS-solution (Amersham) and 0.5 ml methanol. The samples were counted with LKB Rackbeta 1057 instrument using external standard method for the quenching correction. The counting efficiency was about 80%.

Identification of the decarboxylation products. Unlabelled (I) or (II) were incubated with pyridoxal-5'-phosphate (PLP) (each at 1 mM concentration in 5 ml) at 37°, pH 6, for 20 hr. After adjusting the pH to 8 with 0.1 M NaOH the β-carbolines were extracted from the reaction medium with three successive 5 ml volumes of ethyl acetate. The combined organic phases were evaporated to dryness under reduced pressure. Samples from the residues were introduced into the mass spectrometer using a solids insertion probe. Low resolution mass spectra of the compounds were recorded using a Jeol JMS D 300 mass spectrometer with JMA 2000 mass data analysis system at the resolution power of 1000. Ionization current was 300 μA and the electron beam energy was 70 eV. Ionization chamber was maintained at 230°.

Thin layer chromatograms of the extracted incubation products were run using the TLC system described by McIsaac *et al.* [33].

RESULTS

Table 1 summarizes the decarboxylation of (I) and (II) in different media. The data on first two lines indicate that both compounds were degraded significantly faster in the presence of brain homogenate than in buffer, but denaturating pretreatment of the homogenate by heat (for 20 min at 60°) or Hg^{2+} (for 20 min at 37°) did not restore the original level, indicating that the increase was not due to enzyme action. Inclusion of Hg^{2+} actually further enhanced the decarboxylation, and the effect of EDTA (Table 1) suggests that some cations originally present in

Table 1. Decarboxylation of THBC-1-carboxylic acid (I) and 1-methyl-THBC-1-carboxylic acid (II) in different mediums.

Medium	Decarboxylation (pmol CO_2 hr^{-1}) I	II
Buffer	210 ± 16	110 ± 9
Brain homogenate	1390 ± 170*	409 ± 22*
Preheated homogenate	1370 ± 122*	385 ± 27*
Homogenate + 1 mM Hg^{2+}	1780 ± 251*	430 ± 22*
Homogenate + 1 mM EDTA	800 ± 50*	328 ± 34*
Buffer + 0.4 mM PLP	1870 ± 160*	550 ± 30*

* Significantly different ($P < 0.01$, $N = 4$) from the value in the buffer (two-tailed independent Student's t-test).

the homogenate are partly responsible for the accelerated decarboxylation.

Tests with PLP as a potential low-molecular-weight activator suggest that it may be largely responsible for the increased reaction rate. Its effect in the buffer was concentration-dependent (Fig. 3), and 400 μM PLP was sufficient to catalyze the reactions significantly ($P < 0.01$) more than the homogenate (Table 1). When pH was altered between 6.4 and 7.6 the decarboxylation rate in brain homogenate and in 400 μM PLP did not vary significantly ($P > 0.1$) from the values tabulated.

Mass spectrometric examination of the reaction products after incubation of (I) and (II) with PLP indicated that the major reaction products were THBC and 1-methyl-THBC, respectively. Figure 4A shows the spectrum of (I) with the base peak at m/z 171 and the weak molecular peak at m/z 216. Losses of CO_2, CH_2O_2, the latter followed by hydrogen loss yielded other intensive peaks at m/z 172, 170 and 169, respectively. The ion at m/z 143 is formed after decarboxylation by the retro Diels–Alder reaction. After incubation with PLP the base peak of m/z 143 was recorded (Fig. 4B), while the abundance of peak m/z 171 was only 20% and the peak at m/z 172 had abundance of 50%. Peaks at m/z 171 and m/z 169 were significantly lower than in the spectrum of 4A. The molecular peak at m/z 172 and the peak m/z

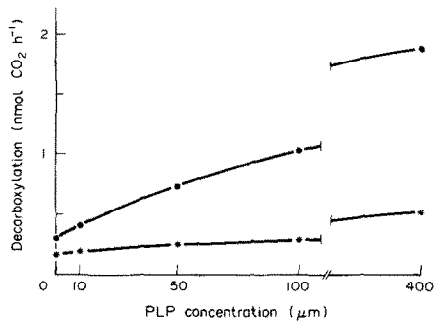


Fig. 3. Effect of pyridoxal-5′-phosphate (PLP) on the decarboxylation of 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (●) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (★) in 100 mM phosphate buffer at 37°, pH 7.4. The points represent means of 4 assays.

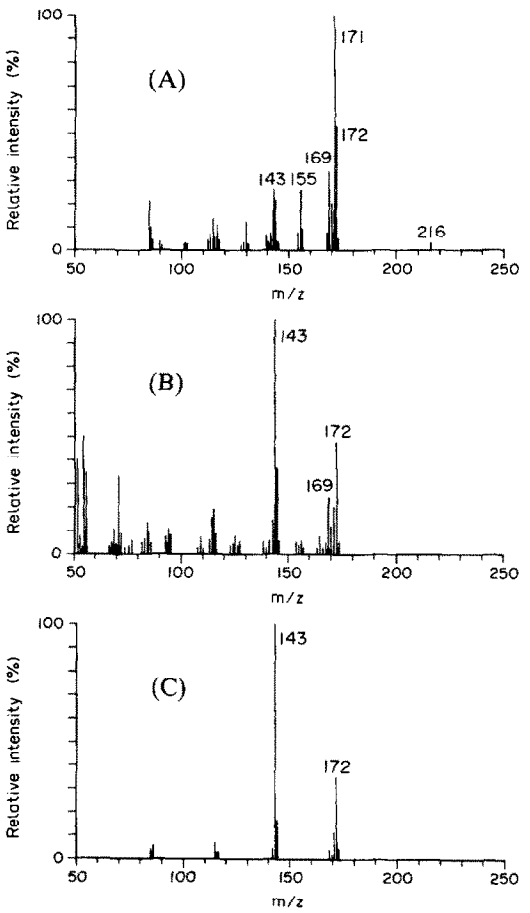


Fig. 4. The mass spectra of an authentic 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (A), a sample from the incubation medium where 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid was incubated with PLP (B), and an authentic THBC (C).

143 in 4B, i.e. the main signals in the spectrum of THBC (Fig. 4C), are the principal indicators for the production of this compound during the incubation. The relative intensities of peaks at m/z 169 and 170 in Fig. 4B suggest the presence of another possible reaction product 3,4-dihydro-β-carboline. Also some unreacted (I) may be present, since there is an intensive peak at m/z 171 (Fig. 4B), while the molecular peak of (I) is no more detectable.

The PLP-treatment altered the base peak of (II) from m/z 185 (Fig. 5A) to m/z 171 (Fig. 5B), which corresponds to that of the presumed product 1-methyl-THBC (Fig. 5C). Another abundant fragment ion is produced by cleavage of CO_2 molecule from (II) yielding a peak at m/z 186. This M^+ peak of 1-methyl-THBC (Fig. 5C) is after incubation more intensive than the peak at m/z 185 (Fig. 5B). The abundance of peak m/z 157 in both Fig. 5B and 5C and its absence from 5A further confirms that 1-methyl-THBC is formed in the incubation. Abundant peaks at m/z 183 and 184 suggest that 1-methyl-3,4-dihydro-β-carboline was also produced.

Data from the TLC analyses were parallel with those of MS. Spots corresponding to (I), (II) and their decarboxylated dihydro and tetrahydro analogs

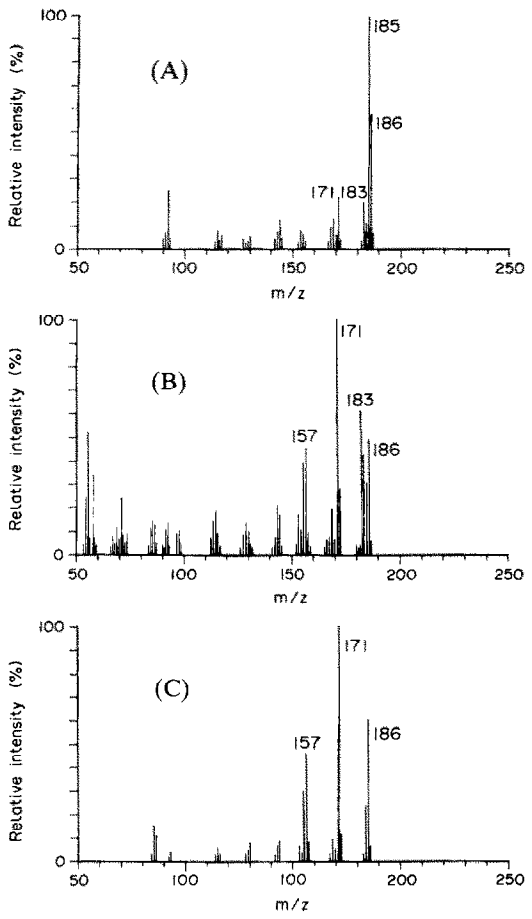


Fig. 5. The mass spectra of an authentic 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (A), a sample from the incubation medium where 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid was incubated with PLP (B), and an authentic 1-methyl-THBC (C).

were observed after the incubations with PLP; the tetrahydro derivatives appearing as main constituents in both cases.

DISCUSSION

Although the results in Table 1 suggest that enzymatic catalysis is not involved in the decarboxylation of (I) or (II), this cannot be completely excluded as the conditions used may be unfavorable for it. In any case, the observed catalytic effect of PLP is sufficient to explain the reported [27] *in vivo* transformations of THBC-1-carboxylic acids and the occurrence of corresponding THBCs in tissues. Results in Table 1 suggest that the decarboxylation is further increased by Hg^{2+} and by ions that could be chelated with EDTA from the brain homogenate. Mechanism of these observed cationic effects remain unknown, but a comparable effect of Cu^{2+} and vanadates on another PLP-dependent non-enzymatic decarboxylation was recently reported [34].

PLP was regarded as the most likely low-molecular-weight effector since it is the principal coenzyme of decarboxylases and it also reacts with primary α-amino carboxylic acids *per se* forming stable compounds through the Schiff's base formation [35]. The present secondary α-amino acids cannot form the Schiff's base (cf. [28, 29]), but rather the postulated cyclic initial complex with PLP (Fig. 6). This mode of action for PLP is supported by our observation (data not included) that neither pyridoxine, pyridoxamine, nor pyridoxamine phosphate had any effect on the process at equal concentrations. The mass spectrometric analyses (Figs. 4 and 5) confirm the production of THBC and 1-methyl-THBC, while identity of the apparent other reaction products remains uncertain. However, formation of 3,4-dihydro analogues of the above products seems quite likely when the present spectra are compared with those of the presumed 3,4-dihydro-β-carbolines run previously under comparable conditions in our laboratory [32, 36]. Formation of alternative products suggests that at least two reaction mechanisms—substitution and elimination—are involved, but the reaction sequences remain open, although a secondary oxidation of the primarily formed THBC is not excluded. The lower decarboxylation rate of (II) is probably due to the steric hindrance of the methyl group in the interaction with PLP.

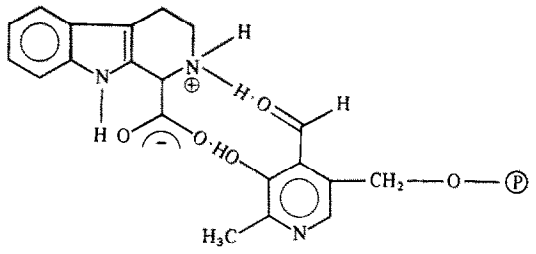


Fig. 6. Scheme of the postulated initial interaction between 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid and pyridoxal-5'-phosphate.

In many studies interest has been focused on the formation of endogenous THBCs from the aldehydes ([23, 24], Fig. 2), although these are highly toxic and barely detectable in free form *in vivo*. The present results do not exclude this possibility, but suggest that if THBC-1-carboxylic acids are formed through the condensation of tryptamine with the two quite abundant physiological metabolites, glyoxylate and pyruvate, they are readily decarboxylated to corresponding THBCs and 3,4-dihydro-β-carbolines under conditions that may be referred to as physiological. Instead of tryptamine, tryptophan may also take part in the condensations and it remains to be studied if these products are also decarboxylated similarly to those described above.

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