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In-vitro decarboxylation of new phenylalanine derivatives

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In a previous paper one of us[1] examined the *in-vitro* action of DOPA decarboxylase on twenty-two tryptophan derivatives. Of the tryptophan derivatives examined only 4-hydroxytryptophan,5-hydroxy-N-acetyltryptophan,5-acetoxy-N-acetyltryptophan together with 5-hydroxytryptophan and 3,4-dihydroxyphenylalanine(DOPA) were found to be decarboxylated using the conventional but not very sensitive Warburg technique.

Table 1. Mammalian decarboxylase: relative rates of decarboxylation of phenylalanine derivatives

Substrate	Decarboxylase	
	guinea pig kidney	mouse brain
3,4-DOPA	100	100
3-hydroxy-4-methoxy-PA	89·3	<10
3-hydroxy-4-methyl-PA	84·5	114
3-methoxy-4-hydroxy-PA	<10	<10
3-methoxy-4-methyl-PA	<10	—
3-methoxy-4-methoxy-PA	<10	<10
2,3-DOPA	90·6	80·5
2-hydroxy-3-methoxy-PA	12·4	20·7
2-methoxy-3-hydroxy-PA	<10	—
2-methoxy-3-methoxy-PA	<10	—
2,4-DOPA	88·2	84·1
2-hydroxy-4-methoxy-PA	46·9	25·8
2-methoxy-4-hydroxy-PA	<10	<10
2-methoxy-4-methoxy-PA	<10	—
2,5-DOPA	83	76·4
2-hydroxy-5-methoxy-PA	<10	—
2-methoxy-5-hydroxy-PA	<10	—
2-methoxy-5-methoxy-PA	<10	—
2,6-DOPA	77·4	39·2
2-hydroxy-6-methoxy-PA	<10	—
2-methoxy-6-methoxy-PA	<10	—
3,5-DOPA	76·3	57
3-hydroxy-5-methoxy-PA	<10	—
3-methoxy-5-methoxy-PA	<10	—
3,4,5-trihydroxy-PA	86·1	46·2
ortho-tyrosine	102	—
meta-tyrosine	100	—
para-tyrosine	<10	—

In the present study, the DOPA decarboxylase activity of mammalian tissues and bacterial L-tyrosine decarboxylase activity was examined on isomers of DOPA and on some new phenylalanine derivatives. The present work, has to be considered as preliminary to further investigations on the possibility that non-biogenic phenylalanine derivatives interfere with the biosynthesis, action and metabolism of endogenous catecholamines.

EXPERIMENTAL

Materials and methods

The following derivatives of DL-phenylalanine (PA) were studied: 3,4-dihydroxy(3,4-DOPA), 3-hydroxy-4-methoxy, 3-hydroxy-4-methyl, 3-methoxy-4-hydroxy, 3-methoxy-4-methyl, 3-methoxy-4-

methoxy; 2,3-dihydroxy(2,3-DOPA), 2-hydroxy-3-methoxy, 2-methoxy-3-hydroxy, 2-methoxy-3-methoxy; 2,4-dihydroxy(2,4-DOPA), 2-hydroxy-4-methoxy, 2-methoxy-4-hydroxy, 2-methoxy-4-methoxy; 2,5-dihydroxy(2,5-DOPA), 2-hydroxy-5-methoxy, 2-methoxy-5-hydroxy, 2-methoxy-5-methoxy; 2,6-dihydroxy(2,6-DOPA), 2-hydroxy-6-methoxy, 2-methoxy-6-methoxy; 3,5-dihydroxy (3,5-DOPA), 3-hydroxy-5-methoxy, 3-methoxy-5-methoxy, 3,4,5-trihydroxy, 2-hydroxy(ortho-tyrosine), 3-hydroxy(meta-tyrosine). 4-Hydroxyphenylalanine(L-para-tyrosine) was also examined. 3,4-DOPA was purchased from Hoffmann-La Roche, Basel and tyrosines from California Foundation for Biochemical Research, Los Angeles. The other compounds were prepared by Dr. C. Pasini in our laboratories. The DOPA decarboxylase enzyme preparations were obtained by centrifugation of homogenates of different tissues (guinea pig kidney and mouse brain) in a Spinco preparative centrifuge at 22,620 *g*. Decarboxylation was carried out in a conventional Warburg apparatus, at 38° and pH 7·4. The flasks contained 4 μmoles of the DL-aminoacid substrate, 50 μg pyridoxal phosphate, 0·07 μmoles phosphate buffer pH 7·4 and 2 ml of the enzyme preparation corresponding to 1 g fresh tissue. The total volume in the flasks was 2·4 ml. Incubation time was $\frac{1}{2}$ hr. Carbon dioxide produced was determined before and after the addition of 0·2 ml of N sulphuric acid.

In the experiment with bacterial L-tyrosine decarboxylase was used, as enzyme preparation, the acetone dried cells of *Streptococcus faecalis R* and followed the Warburg method of Sloane-Stanley[2] for dried cells grown in a vitamin B_6-free medium.

RESULTS

Tables 1 and 2 show the relative rates of decarboxylation of DOPA isomers and phenylalanine derivatives. In the experiments with guinea pig kidney and mouse brain the 80–100% of the theoretical

TABLE 2. BACTERIAL L-TYROSINE DECARBOXYLASE: RELATIVE RATES OF DECARBOXYLATION OF PHENYLALANINE DERIVATIVES

Substrate	
para-tyrosine	100
meta-tyrosine	75
ortho-tyrosine	5
3,4-DOPA	20
3-hydroxy-4-methoxy-PA	<5
3-hydroxy-4-methyl-PA	<5
3-methoxy-4-hydroxy-PA	<5
3-methoxy-4-methyl-PA	<5
3-methoxy-4-methoxy-PA	<5
2,3-DOPA	30
2-hydroxy-3-methoxy-PA	<5
2-methoxy-3-hydroxy-PA	<5
2-methoxy-3-methoxy-PA	<5
2,4-DOPA	60
2-hydroxy-4-methoxy-PA	<5
2-methoxy-4-hydroxy-PA	<5
2-methoxy-4-methoxy-PA	<5
2,5-DOPA	<5
2-hydroxy-5-methoxy-PA	<5
2-methoxy-5-hydroxy-PA	—
2-methoxy-5-methoxy-PA	<5
2,6-DOPA	<5
2-hydroxy-6-methoxy-PA	—
2-methoxy-6-methoxy-PA	—
3,5-DOPA	5
3-hydroxy-5-methoxy-PA	—
3-methoxy-5-methoxy-PA	<5
3,4,5-trihydroxy-PA	25

amount of carbon dioxide was formed from the substrate DL-3,4-DOPA. From one molecule of DL aminoacid half a molecule of carbon dioxide is formed, as it is known that the decarboxylases are stereospecific and form carbon dioxide only from L-isomer. In the experiments with bacterial L-tyrosine decarboxylase the 90% of the theoretical amount of carbon dioxide was formed from the substrate L-para-tyrosine within 3 hr.

Data of Tables 1 and 2 are not to be considered as biological constants; they however show the relative susceptibility of the compounds to enzyme action. Both mammalian decarboxylases act at good rates on all the DOPA isomers, 3-hydroxy-4-methylphenylalanine and 3,4,5-trihydroxyphenylalanine. Strangely enough 3-hydroxy-4-methoxyphenylalanine is decarboxylated only by guinea pig's enzyme. 2-hydroxy-4-methoxyphenylalanine is a poor substrate for both decarboxylases. All other compounds of Table 1 show a rate of decarboxylation less then 10% of that of DOPA. Our method does not allow us to decide whether some of these compounds are substrates for the mammalian enzymes. Para-tyrosine is the best substrate for bacterial L-tyrosine decarboxylase. Compared with para-tyrosine, DOPA isomers and tyrosine isomers are decarboxylated at lower rates in the following decreasing order: metatyrosine, 2,4-DOPA, 2,3-DOPA, 3,4-DOPA, ortho-tyrosine and 3,5-DOPA. L-tyrosine decarboxylase is inert towards 2,5-DOPA and 2.6-DOPA. 3,4,5-trihydroxyphenylalanine is a fairly good substrate for the bacterial enzyme while 3-hydroxy-4-methylphenylalnine is not decarboxylated by L-tyrosine decarboxylase.

DISCUSSION

Our observations on the substrate specificity of bacterial L-tyrosine decarboxylase of *Streptococcus faecalis* and L-DOPA decarboxylase of mammalian tissues are in accordance with previous observations of other authors who used the Warburg technique.[3–10] As expected, mammalian DOPA decarboxylase decarboxylates ortho and meta-tyrosine but not para-tyrosine while bacterial L-tyrosine decarboxylase decarboxylates only para- and meta-tyrosine. All the isomers of DOPA, including 2,6-DOPA, are good substrates of L-DOPA decarboxylase while 3,4-DOPA, 2,3-DOPA and 2,4-DOPA are the only isomers of DOPA significantly decarboxylated by bacterial L-tyrosine decarboxylase.

Our results give some new informations on the substrate specificity for decarboxylase activity. The compounds having two methoxyl groups instead of two hydroxyl groups in the benzene ring are not decarboxylated by neither decarboxylases. The introduction of one methoxyl group in the benzene ring of *p*-, *m*-, and *o*-tyrosine modifies the susceptibility of these compounds to the decarboxylating activity of mammalian and bacterial decarboxylases. Bacterial decarboxylase does not decarboxylate any compound having both an hydroxyl and a methoxyl group in the benzene ring even when the hydroxyl group is placed in para- or in meta- position. Mammalian decarboxylase, on the contrary, decarboxylates at good rate the compounds having the methoxyl group in para-position. The presence of the methoxyl group in any other position in the benzene ring strongly reduces or prevents the decarboxylation even when the hydroxyl group is in meta- or ortho-position.

Carlsson *et al.*,[11] Mitoma *et al.*[12] and Chrusciel[13] showed that aminoacid drugs like DOPA, *m*- and *o*-tyrosine produced pharmacological effects of their own or through their corresponding amines. Recently Day and Rand[14] suggested that α-methyl-DOPA can serve as precursor of α-methyl noradrenaline in the body. The α-methyl noradrenaline may enter noradrenaline storage sites and then be released as a false neuro-transmitter. It would be of interest to know whether one of our new synthetic aminoacids which were found to be acted upon by mammalian decarboxylase (that are 3-hydroxy-4-methoxyphenylalanine, 3-hydroxy-4-methylphenylalanine, 2-hydroxy-3-methoxyphenylalanine, 2-hydroxy-4-methoxyphenylalanine and 3,4,5-trihydroxyphenylalanine) produces direct pharmacological effects or works as false neuro-transmitter as suggested by Day and Rand for α-methyl-DOPA.

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Curare protection against succinylcholine action on muscle

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IT HAS been reported by Ochs *et al.*[1] that succinylcholine applied to frog sartorius muscle greatly reduces twitch heights in response to single direct stimuli. Further, it was shown that curare pretreatment blocks the action of succinylcholine, and it was also found that succinylcholine produces a generalized decrease in resting membrane potentials that is not restricted to end-plate regions. Katz and Miledi[2] have criticized this work and have suggested that the decrease in twitch height observed by Ochs *et al.* may have been due to inadequate direct stimulation or to conduction block at depolarized junctions. Katz and Miledi found that the primary depolarizing action of succinylcholine is restricted to the motor end plates. Further, they found that the effect of succinylcholine on the twitch response is only temporary and follows approximately the same time course as block of the tibial conducted spike when the frog sartorius muscle is directly stimulated on the pelvic end. This indicates that the decreased contractile response is due to a conduction block at the temporarily depolarized end plates. Katz and Miledi did not try curare pretreatment because they felt that there was no general muscle action of succinylcholine to antagonize. The following experiments were undertaken to test possible antagonism by curare of the temporary succinylcholine action.

Frog sartorius muscles, from winter *Rana pipiens*, were stretched to 120% of their 'zero tension' length. The muscles were completely immersed in phosphate-buffered Ringer's solution (with or without curare and/or succinylcholine, pH 7·2, temperature 20° ± 0·4°) and stimulated by two silver electrodes in the solution, one electrode being near each end of the muscle. A square-wave stimulus of 1 msec and 135 V was used. Most of the muscle fibers must have been receiving a direct stimulus, since added curare had only a slight effect on twitch strength. Single isometric twitches were recorded by means of strain gages mounted on a brass torsion bar and a standard amplifying and recording system.

The results (Fig. 1) confirm the finding of Ochs *et al*[1]. that curare protects against the decrease in contractile strength produced by succinylcholine. However, these authors presented data only for the first 10 or 15 min after exposure to succinylcholine. Had they continued, they probably would have seen the recovery observed here. This indicates that the interpretation of Katz and Miledi was probably correct and is consistent with the finding in cat gracilis muscle that curare pretreatment abolishes the end-plate region block to propagation of a directly excited muscle action potential, such temporary block being produced by decamethonium or acetylcholine.[3] It is interesting to note