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PHARMACOLOGY OF SOME INHIBITORS OF AROMATIC AMINO-ACID DECARBOXYLASE

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UDC 615.355:577.152.411.042.2

The sodium salts of D,L-β-(3,4-dihydroxyphenyl)lactic (I) and D,L-β-(5-hydroxyindolyl-3)lactic acids (II), and also L-α-methyldopa (AMD) are competitive inhibitors of aromatic amino-acid decarboxylase (AAAD). Unlike AMD, compounds I and II are not substrates for AAAD. Compound II selectively inhibits the decarboxylation of L-5-hydroxytryptophan. Compound I and AMD potentiate excitation induced in mice by L-dopa but do not affect excitation induced by L-5-hydroxytryptophan. Compound II weakens excitation induced by both L-dopa and L-5-hydroxytryptophan. Pyridoxin hydrochloride and pyridoxal phosphate weaken excitation induced by L-dopa and L-5-hydroxytryptophan. Compound I and AMD abolish this action of the B_6 vitamins.

KEY WORDS: D,L-β-(3,4-dihydroxyphenyl)lactic acid; D,L-β-(5-hydroxyindolyl-3)lactic acid; L-3,4-dihydroxyphenylalanine; L-5-hydroxytryptophan; L-α-methyldopa; pyridoxin.

Aromatic amino-acid decarboxylase (AAAD; EC 4.1.1.26 or 28) is a pyridoxal enzyme which forms dopamine from L-3,4-dihydroxyphenylalanine (L-dopa) or serotonin from L-5-hydroxytryptophan (L-5-HT). Known inhibitors with the action of carbonyl poisons inhibit both reactions [7]. The search for compounds capable of inhibiting one of the two reactions selectively and the study of their pharmacological properties are of theoretical interest.

In the investigation described below sodium salts of D,L-β-(3,4-dihydroxyphenyl)lactic acid (I) and D,L-β-(5-hydroxyindolyl-3)lactic acid (II) were studied as inhibitors. These substances are the hydroxy analogs of L-dopa and L-5-HT respectively (see the formulas). The hydroxyl group replacing the amino group prevents the compound from irreversibly inactivating the carbonyl group of pyridoxal. The factor of structural similarity with one of the substrates may in this case play a decisive role in the selectivity of action of the substances depending on the substrate used.

EXPERIMENTAL METHOD

The inhibitory effect of compounds I and II on AAAD was tested *in vitro* by determining the activity of the enzyme by a manometric method in Warburg's apparatus. The known substrate-like inhibitor L-α-methyldopa (AMD) [4] was used for comparison.

The decarboxylase was isolated from rabbit kidney, a tissue in which the enzyme has high specific activity [9]. The animals were decapitated and the kidneys homogenized with glass sand in the cold for 20 min in 0.067 M phosphate buffer, pH 6.8. The buffer was added in a volume of 1.5 ml per gram of kidneys. The homogenate was centrifuged for 20 min at 2000g and the supernatant was then centrifuged for 60 min at 100,000g. Samples of 1.5-2 ml of the transparent supernatant were taken. The incubation sample, in a volume of 3 ml, contained:

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 0007-4888/78/8610-1334$07.50

TABLE 1. Comparative Inhibitory Activity of Compounds I, II, and AMD on AAAD

Inhibitors in concentration of 1×10^{-3} M	Degree of reduction in rate of decarboxylation in presence of inhibitors	
	L-dopa, 2×10^{-3} M	L-5-HT, 4×10^{-3} M
I	2,75	2,99
II	1,67	6,26
AMD	1,48	3,31

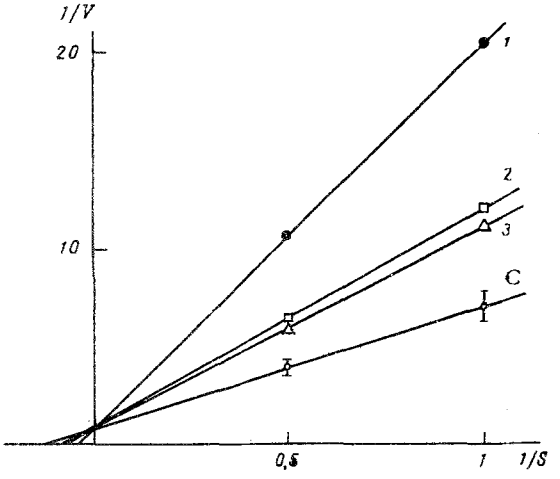


Fig. 1

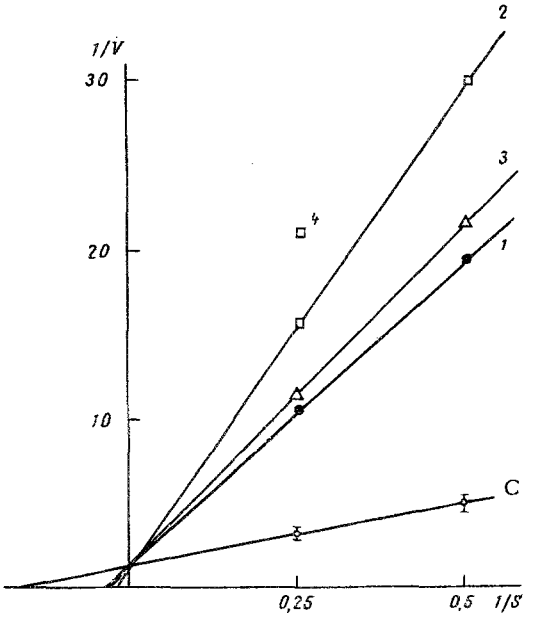


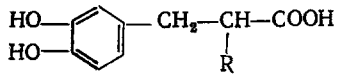
Fig. 2

Fig. 1. Activity of AAAD as a function of substrate (L-dopa) concentration in control (C) and in presence of inhibitors (1-3). Lineweaver—Burk reciprocal plot. Abscissa, reciprocal of final concentration (1/S) of L-dopa (in mM); ordinate, reciprocal of reaction velocity (1/V) expressed as number of micromoles CO_2 liberated per minute. 1) Compound I; 2) compound II; 3) AMD. Concentration of inhibitors $1\cdot10^{-3}$ M.

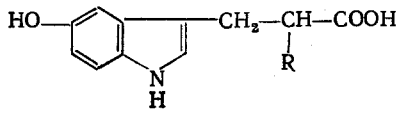
Fig. 2. AAAD activity as a function of substrate (L-5-HT) concentration in control (C) and in presence of inhibitors (1-3). Lineweaver—Burk reciprocal plot. Ordinate, reciprocalof reaction velocity (1/V) expressed as number of micromoles CO_2 liberated per minute. Abscissa, reciprocal of final concentration (1/S) of L-5-HT, in mM. 1) Compound I in concentration of $1\cdot10^{-3}$ M; 2) compound II in concentration of $1\cdot10^{-4}$ M; 3) AMD in concentration of $1\cdot10^{-3}$ M; 4) compound II in concentration of $1\cdot10^{-3}$ M.

pyridoxal phosphate ($1\cdot10^{-4}$ M), L-dopa ($2\cdot10^{-3}$ M) or L-5-HT ($4\cdot10^{-3}$ M), and compounds I, II, or AMD in concentrations of $1\cdot10^{-4}$ M to $2\cdot10^{-3}$ M. All concentrations are final. The substances were dissolved in 0.067 M phosphate buffer, pH 6.8, and in the case of L-dopa, AMD, and L-5-HT, during heating to 80°C. The activity of the enzyme was determined from the quantity of CO_2 liberated per minute during the first 10 min of the reaction. The preincubation time was 15 min, the temperature 37°C, and air in the side tubes of the Warburg apparatus was replaced by argon. (See scheme, top following page.)

L-5-HT is known to be an inferior substrate to L-dopa [8]. It was therefore used in higher concentration and with a higher concentration of the enzyme to produce the same output of CO_2 in the control, whatever the substrate used. The character of inhibition was determined from a Lineweaver—Burk reciprocal plot.



where R = NH_2 represents L-3,4-dihydroxyphenylalanine (L-dopa); where R = OH represents D,L-β-(3,4-dihydroxyphenyl)lactic acid.



where R= NH_2 represents L-5-hydroxytryptophan (L-5-HT); where R= OHrepresents D,L-β-(5-hydroxyindolyl-3)-lactic acid.

The effect of compounds I, II, and AMD on excitation induced by L-dopa and L-5-HT was investigated in mice. L-dopa or L-5-HT was injected intraperitoneally into mice divided into groups of six. The mice were kept in a crowded box measuring 20 × 10 × 7 cm. The surrounding temperature was maintained at 25°C. After a dose of 400 mg/kg severe psychomotor excitation developed. Animals receiving L-dopa were characteristically aggressive and exhibited peripheral sympathomimetic effects [2], whereas those receiving L-5-HT exhibited movements simulating an orienting reaction and developed diarrhea [3, 6]. In both cases, as a result of intensified muscular work, increased perspiration, or diarrhea, a loss of weight was observed and this was used as the criterion for quantitative evaluation of the intensity of excitation. The mice were weighed a second time 2 h after receiving L-dopa and L-5-HT with an accuracy of 10 mg. The action of L-dopa and L-5-HT in the control and after administration of compounds I, II, or AMD 30 or 60 min beforehand in doses of between 50 and 500 mg/kg was compared. Experiments were carried out on mice kept on a Larsen I standard diet, and also on mice receiving additional vitamins B_6 for 1 to 7 days: pyridoxal phosphate in a dose of 1 mg/kg twice a day intraperitoneally and pyridoxin hydrochloride added to the daily drinking water in a dose of 50 mg/kg.

EXPERIMENTAL RESULTS

The compounds studied are competitive inhibitors of AAAD. They inhibited the decarboxylation of L-5-HT more strongly than that of L-dopa (Table 1). The marked selectivity of the hydroxy analogs I and II, depending on the substrate used in the reaction, will be noted. In the presence of L-dopa compound I had greater inhibiting activity (Fig. 1), whereas in the presence of L-5-HT compound II was more active (Fig. 2). As regards AMD, no appreciable selectivity of action was found for this compound. Unlike AMD, the hydroxy analogs themselves are not substrates for AAAD.

The hydroxy analogs and AMD had an inhibitory effect on the CNS expressed as a reduction in the motor activity of the mice and hypothermia. However, when given in conjunction with L-dopa and L-5-HT, significant differences were found between the action of the compounds.

Compound I and AMD potentiated psychomotor excitation induced by L-dopa. As a result the loss of weight of the mice was greater after combined administration of the compounds than the control effect of L-dopa alone (Table 2). Meanwhile, compound I did not affect excitation induced by L-5-HT, whereas AMD weakened it.

Compound II weakened the central effects of L-dopa and L-5-HT. With a dose of 500 mg/kg, corresponding to 250 mg/kg of the active L-form, both the central and peripheral effects of L-dopa and L-5-HT were virtually completely abolished.

Preliminary administration of pyridoxin and pyridoxal phosphate weakened the action of L-dopa and L-5-HT. Compound I and AMD, given after this premedication, but not compound II, abolished the "pyridoxal protection" and restored the central effects of L-dopa but not of L-5-HT, in the form in which they were observed in animals not receiving supplementary vitamins B_6. The results show that the hydroxy analogs are typical substrate-like inhibitors of AAAD, with a selective action depending on the substrate participating in the reaction. The fact that the hydroxy analogs are not substrates for AAAD is of considerable importance for the selectivity of their inhibitory action. This feature significantly distinguishes the hydroxy analogs from AMD. The universality of the inhibitory action of AMD can be explained on the grounds that AMD is a quasi-substrate for AAAD [5]. Its inhibitory effect is of the same nature as the competitive relations existing between the two natural substrates L-dopa and L-5-HT, each of which is able to inhibit the decarboxylation of the other [10].

TABLE 2. Effect of Compounds I, II, and AMD on Loss of Weight of Mice Caused by Administration of L-dopa or L-5-HT

Premedication 1 h beforehand (substance and dose, mg/kg)	Under ordinary conditions				After administration of pyridoxal phosphate and pyridoxin for 4 days			
	L-dopa, mg/kg		L-5-HT, mg/kg		L-dopa, mg/kg		L-5-HT, mg/kg	
	200	400	200	400	200	400	200	400
Physiological saline (control)	0,39±0,1	0,92±0,09	0,38±0,02	0,72±0,06	0,1±0,02	0,54±0,09	—	0,2±0,04
I:								
100	0,42±0,06	0,96±0,08	0,4±0,03	0,78±0,08	0,4±0,07	1,1±0,07	—	—
200	0,64±0,07	1,21±0,12*	0,37±0,04	0,69±0,1	0,57±0,06	D	—	0,15±0,06
II:								
100	—	0,6±0,07	—	0,65±0,07	—	0,3±0,05	—	—
200	—	0,35±0,05	—	0,46±0,05	—	—	—	—
500	—	—	—	0,13±0,03	—	—	—	—
AMD:								
50	0,71±0,04	1,1±0,09	0,29±0,08	0,83±0,02	0,46±0,06	0,94±0,06	—	0,36±0,06
100	0,88±0,07	1,3±0,09*	0,21±0,08	0,62±0,06	0,85±0,05	1,2±0,07*	—	0,46±0,04

Legend. 1. Mean values of decrease in body weight of mice, in g, 2 h after injection of L-dopa or L-5-HT shown in Table. 2. Groups of mice weighed a second time, and which still survived by the end of the second hour after injection of L-dopa marked by asterisk. 3. D — most animals died before end of 2 h; a blank space indicates no change in body weight.

The different biochemical characteristics of the compounds determine their different pharmacological properties. Excitation induced by L-dopa and L-5-HT reflects the action of the dopamine and serotonin formed from them, respectively. It can accordingly be concluded that the effects of compound II correspond fully to the characteristics of an AAAD inhibitor. By preventing the formation of biogenic amines, compound II weakened the action of their immediate precursors. Meanwhile compound I and AMD not only did not weaken the action of L-dopa, but actually potentiated it. Pyridoxal enzymes participate not only in the decarboxylation of exogenous L-dopa but also in reactions which form part of the mechanism of physiological inactivation of L-dopa. After combined administration of L-dopa and vitamins B_6, the latter did not so much facilitate the formation of functionally active dopamine as hasten its physiological inactivation [1]. This is the mechanism of weakening of the effects of L-dopa when given after preliminary administration of vitamins B_6. Presumably, with their structural similarity to L-dopa, compound I and AMD can compete with it in all reactions involving the participation of pyridoxal phosphate. Inhibition of the pyridoxal mechanisms of inactivation of exogenous L-dopa evidently lies at the basis of the strengthening of the central action of exogenous L-dopa under the influence of these compounds.

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