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L-Dihydroxyphenylalanine: Effect on Levels of Free Amino Acids in Rat Liver[1] (38302)

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L-Dihydroxyphenylalanine (L-Dopa) is a neutral amino acid administered in large doses to patients with Parkinson's disease (1, 2). It is anticipated that long term therapy with L-Dopa might interfere with the normal metabolism of other amino acids by competition for common pathways of decarboxylation (3), transamination (4), and intestinal (5), renal tubular (6), blood to brain (7) transport and protein synthesis (8). We previously observed that when L-Dopa was administered orally to parkinsonian patients 0.5 hr before phenylalanine and tyrosine tolerance tests, the plasma levels of these amino acids were decreased (9). These results suggest that L-Dopa competes with phenylalanine and tyrosine for a common intestinal transport pathway. L-Dopa also alters the concentration of certain brain amino acids. Chronic subcutaneous injections of L-Dopa increased brain methionine levels and decreased glutamine in the rat (10). In the present study, we have determined the effects of L-Dopa administration on hepatic-free amino acids in the rat.

Materials and Methods. Two groups of six male Sprague–Dawley rats (180–220 g) were injected subcutaneously with either 500 mg/kg or 1000 mg/kg of L-Dopa daily for 8 days. The L-Dopa was administered as a suspension in isotonic saline (either 100 mg or 200 mg/ml) which was homogenized in a Potter–Elvejhem homogenizer prior to injection. A group of six control rats which received an equivalent volume of isotonic saline was used with each L-Dopa group. The rats were fed *ad lib* with a standard Purina Lab Chow (Ralston Purina Co., Wilbrahan, MA) diet. The rats were weighed and then decapitated 2 hr after the final injection of either the L-Dopa suspensions or saline. The control and the L-Dopa-treated rats were housed in the same room under identical lighting conditions and killed at the same time of day to avoid differences due to the diurnal variation in hepatic amino acids. The whole livers were rapidly removed, weighed and a small aliquot saved for protein determination by the method of Lowry *et al.* (11). The remainder of the liver was homogenized in a ground glass homogenizer with 10 vol of 6% (w/v) perchloric acid at 4°.

The free amino acids were extracted from the liver homogenate by the method described by Saifer (12). Amino acids were measured with a Jeol JLC-5AH automatic amino acid analyzer by a modification of the method of Spackman *et al.* (13). The resins used were Jeolco AR-15 for basic amino acids (0.8 × 14-cm column) and Jeolco AR-50 for neutral and acidic amino acids (0.8 × 52-cm column). Elution of the short column with 0.38 *N* sodium citrate buffer (pH 4.16), temperature 35°, yielded tyrosine and phenylalanine as one peak followed by ammonia, lysine and histidine. Tryptophan and arginine were eluted from the same column with 0.35 *N* sodium citrate buffer (pH 5.36), temperature 55°. Acidic and neutral amino acids were separated on the long column using lithium citrate buffer as described in the Jeol Manual (Jeol, U.S.A., Inc., Cranford, NJ).

Results. The effect of subcutaneous L-Dopa injections on changes in body weight, liver weight and liver protein are shown in Table I. 500 mg/kg L-Dopa prevented the normal 24% weight gain of the animals and an 8% decrease in body weight was observed at a dose of 1000

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TABLE I.[a]

	% Change in body weight	Liver weight (g)	Protein mg/g liver	Protein mg/liver
Control	+ 24 ± 4	11.4 ± 0.7	174 ± 6	1975 ± 127
L-Dopa (500 mg/kg)	+ 1 ± 3***	8.3 ± 0.3*	183 ± 5	1506 ± 63**
L-Dopa (1000 mg/kg)	− 8 ± 7*	6.9 ± 0.6***	185 ± 10	1259 ± 69*

* $P < 0.005$. ** $P < 0.02$. *** $P < 0.001$.

[a]Effect of L-Dopa on body weight, liver weight and protein content of the rat liver. The values are the mean ± SE of five to six rats in each group. *—*** (control versus L-Dopa-treated).

mg/kg. The liver weight as well as the total protein content of the liver was similarly decreased after chronic L-Dopa administration. L-Dopa had no significant effect on the amount of protein per grain of liver, suggesting that dehydration was probably not a major factor in the decrease in liver weight.

The free amino acid content in the control rat livers were generally within the range of previously published values (14–16). Comparison of the levels of amino acids in the livers of control rats and rats treated with 500 mg/kg of L-Dopa indicated that daily subcutaneous injections of this drug significantly increased the concentrations of isoleucine, methionine, threonine and tyrosine (Table II). 1000 mg/kg of L-Dopa produced an even greater increase in the concentration of tyrosine and threonine in the liver as well as inducing a significant increase in hepatic–aspartic acid and serine (Table III). The increase in methionine observed with 500 mg/kg was not significant at the dose of 1000 mg/kg. Similarly, isoleucine was not elevated by 1000 mg/kg of L-Dopa. Only hepatic glutamic acid showed a significant decrease after L-Dopa treatment (1000 mg/kg) (Table III). The remaining amino acids listed in Tables II and III were not significantly altered by the treatment with L-Dopa and L-Dopa was not detectable in the livers of either control or treated rats.

Discussion. The concentration of amino acids in the liver are regulated by (a) intestinal absorption (b) rate of uptake and release from the hepatocyte, (c) rate of utilization in protein synthesis or gluconeogenesis (d) rate of breakdown or synthesis by transamination and (e) rate of amino acid release during protein catabolism. L-Dopa may alter one or more of these metabolic pathways.

We previously presented data suggesting that L-Dopa competes with phenylalanine and tyrosine for intestinal transport pathways in man

TABLE II. Liver-Free Amino Acid Concentration (μmoles/g wet wt of liver).[a]

	Controls	L-Dopa (500 mg/kg)	% of control
Alanine	1.580 ± 0.116	1.375 ± 0.111	87
Arginine	0.027 ± 0.003	0.027 ± 0.002	100
Aspartic acid	0.401 ± 0.034	0.467 ± 0.042	116
Glycine	1.645 ± 0.042	1.709 ± 0.064	104
Histidine	0.280 ± 0.018	0.227 ± 0.031	81
Isoleucine	0.123 ± 0.010	0.167 ± 0.010***	136
Leucine	0.276 ± 0.025	0.279 ± 0.009	101
Lysine	0.277 ± 0.025	0.290 ± 0.034	105
Methionine	0.106 ± 0.009	0.154 ± 0.013**	145
Phenylalanine	0.076 ± 0.006	0.073 ± 0.010	96
Proline	0.142 ± 0.012	0.123 ± 0.004	87
Serine	0.263 ± 0.023	0.336 ± 0.033	128
Threonine	0.160 ± 0.012	0.257 ± 0.030**	161
Tryptophan	0.030 ± 0.002	0.030 ± 0.001	100
Tyrosine	0.076 ± 0.007	0.104 ± 0.008*	137
Valine	0.225 ± 0.014	0.228 ± 0.010	101

* $P < 0.025$. ** $P < 0.02$. *** $P < 0.01$.

[a]The values are expressed as the mean ± SE.

TABLE III. Liver Free Amino Acid Concentration (μmoles/g wet wt of liver).[a]

	Controls	L-Dopa (1000 mg/kg)	% of control
Alanine	1.815 ± 0.103	1.548 ± 0.085	85
Arginine	0.028 ± 0.002	0.030 ± 0.005	107
Aspartic acid	0.442 ± 0.022	0.636 ± 0.032***	144
Glutamic acid	2.022 ± 0.175	1.453 ± 0.107*	72
Glycine	1.789 ± 0.086	1.592 ± 0.119	89
Histidine	0.329 ± 0.021	0.255 ± 0.027	78
Isoleucine	0.119 ± 0.006	0.119 ± 0.008	100
Leucine	0.230 ± 0.012	0.209 ± 0.014	91
Lysine	0.300 ± 0.008	0.358 ± 0.040	119
Methionine	0.108 ± 0.018	0.144 ± 0.018	133
Phenylalanine	0.068 ± 0.005	0.073 ± 0.006	107
Proline	0.159 ± 0.009	0.144 ± 0.012	91
Serine	0.226 ± 0.009	0.462 ± 0.021****	204
Threonine	0.191 ± 0.018	0.348 ± 0.027***	182
Tryptophan	0.031 ± 0.000	0.031 ± 0.002	100
Tyrosine	0.072 ± 0.008	0.110 ± 0.009**	153
Valine	0.213 ± 0.015	0.217 ± 0.021	102

* $P < 0.025$. ** $P < 0.02$. *** $P < 0.005$. **** $P < 0.001$.
[a]The values are expressed as the mean ± SE.

(9). Since hepatic phenylalanine levels did not change during L-Dopa treatment, and tyrosine levels actually increased in the rats, it seems unlikely that L-Dopa significantly interferes with the uptake of amino acids by the hepatocyte in the present experiments. Under similar experimental conditions, L-Dopa (1000 mg/kg) did not compete with other amino acids for transport into the brain (10).

There is evidence suggesting that L-Dopa may interfere with protein synthesis perhaps by inhibiting the binding of other amino acids to their specific transfer RNAs. Weiss *et al.* (17) observed that L-Dopa (500 mg/kg) injected intraperitoneally in rats produced disaggregation of brain polysomes. Disaggregation of polysomes is usually evidence of disturbed protein synthesis. Furthermore, L-Dopa inhibited protein, glycoprotein, and RNA and DNA synthesis in rat brain mitochondria and mouse leukemia cells (8). The synthesis of two liver enzyme proteins, dopa decarboxylase (18) and tyrosine transaminase (4, 19) are inhibited by L-Dopa injections in rats. The decrease in tyrosine transaminase may contribute to the increase in liver tyrosine concentration since this enzyme regulates the major pathway of tyrosine catabolism. The decrease in total liver protein and the increase in isoleucine, methionine, threonine, tyrosine, aspartic acid and serine during L-Dopa treatment in our experiments are consistent with an L-Dopa-induced inhibition of protein synthesis. Decreased protein synthesis could alter the amino acid pool composition, resulting in an elevation in those amino acids which are not metabolized rapidly by other pathways. However, we are unable to explain the isoleucine and methionine increase after 500 mg/kg but not after 1000 mg/kg.

Other studies on animals administered excessive amounts of an individual amino acid (20, 21) also demonstrate a depression in body weight which varies with each amino acid (22) and may or may not be associated with a depression of food intake. It seems unlikely that an L-Dopa-induced decrease in food intake could entirely explain our results since the changes in rat liver-free amino acids produced by starvation are entirely different from our findings (14, 23). In the livers of rats starved for 8 days, the concentrations of alanine, threonine, glycine, and methionine were decreased and valine, isoleucine and leucine were increased (14).

Summary. The concentrations of liver free amino acids in L-Dopa-treated and control rats were assayed by a Jeol amino acid analyzer. Subcutaneous injections of 500 mg/kg L-Dopa daily for 8 days increased the concentrations of isoleucine, methionine, threonine and tyrosine. 1000 mg/kg L-Dopa produced an even greater increase in the concentration of tyrosine and threonine in the liver as well as inducing a sig-

nificant increase in hepatic–aspartic acid and serine. Only glutamic acid decreased after chronic L-Dopa treatment (1000 mg/kg). These effects of L-Dopa on hepatic-free amino acid levels are discussed with reference to some of the known metabolic actions of this drug.

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