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INHIBITION OF DOPA DECARBOXYLASE BY THE HYDRAZINO ANALOG OF α-METHYLDOPA

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Abstract—DL-α-Methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid (HMD) is a potent inhibitor of DOPA decarboxylase *in vitro*. *In vivo*, the hydrazino acid inhibited the accumulation of serotonin in the kidneys of mice given 5-hydroxytryptophan (5-HTP); it inhibited the urinary excretion of serotonin by 5-HTP-dosed rats; it decreased the rate of expiration of radioactive CO_2 by rats which were dosed with L-1-^{14}C-tyrosine, phenylalanine or α-methyldopa. However, it did not affect the rate of expiration of ^{14}C-O_2 by rats which were dosed with DL-1-^{14}C-glutamic acid. HMD was concentrated by the kidneys in rats, but did not enter the brain to a measurable extent, and did not affect concentrations of endogenous serotonin in brain. It was itself decarboxylated in the rat to a greater extent after oral than after intraperitoneal administration. HMD apparently did not inhibit monoamine oxidase *in vivo*.

THE INHIBITION of L-DOPA decarboxylase by numerous types of compounds has been reviewed by Clark.[1] It is now well known that carbonyl trapping agents which can react with pyridoxal, or its phosphate, inhibit decarboxylation. Also certain amines and amino acids may react with these aldehydes to form Schiff bases and possibly isoquinoline derivatives.[2, 3] Murphy and Sourkes[4] have shown recently that several derivatives of α-methylphenylalanine inhibit the conversion of DOPA to dopamine *in vivo*.

We wish to describe a new DOPA decarboxylase inhibitor that is more potent than any now available. The compound, DL-α-methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid (HMD),* is closely related to α-methyldopa, a known DOPA decarboxylase inhibitor.[5] HMD is also a derivative of hydrazine.

EXPERIMENTAL AND RESULTS

Inhibition of L-*DOPA decarboxylase* in vitro

Conventional anaerobic manometric procedures were followed. The source of enzyme was a lyophilized aqueous extract of hog kidneys reconstituted (100 mg/ml) in 0·067 M phosphate buffer, pH 6·8. Warburg flasks were set up to contain the following: main compartment, 0·2 ml pyridoxal phosphate solution† (0·2 μmole), 0·3 ml enzyme, 0·3 ml inhibitor solution, and 1·7 ml 0·067 M phosphate buffer, pH 6·8; side arm one, 0·3 ml L-DOPA solution (10 μmole); side arm two, 0·2 ml 2 N sulfuric acid.

* The compound was supplied by F. W. Bollinger, M. Sletzinger, and J. M. Chemerda, Merck Sharp & Dohme Research Laboratories, Rahway, N.J. A description of its synthesis will be published elsewhere.

† California Corp. for Biochemical Research.

TABLE 1. INHIBITION OF DOPA DECARBOXYLASE

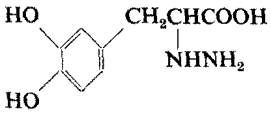
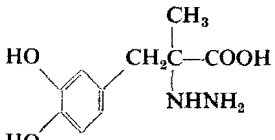
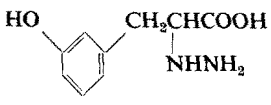
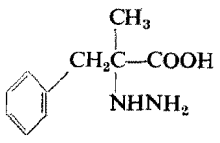
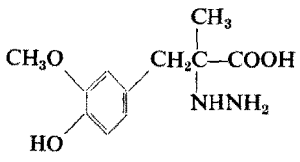
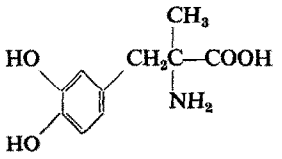
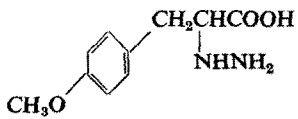
Compound*	*In vitro*				*In vivo*			
	Amount in flask† (μmole)	Inhibition (%)	Approximate I_{50}‡ (μmole)	Relative molar potency§	No. of groups	ED_{50}¶ (mg/kg)	95% confidence limits	Relative molar potency§
	0·0002 0·002 0·02	30 72 94	0·0006	1500	10	0·061	0·032, 0·099	77
 (HMD)	0·0002 0·002 0·02	17 85 97	0·0006	1500	10	0·088**	0·080, 0·096	57
	0·0002 0·002 0·02	31 50 68	0·002	450	9	0·192	0·094, 0·391	23
	0·02 0·20 2·00	29 77 91	0·055	16	4	2·0		2
	0·20	83	0·045	20	4	3·0		2

TABLE 1.—*continued.*

Compound*	*In vitro*				*In vivo*			
	Amount in flask† (μmole)	Inhibition (%)	Approximate I_{50}‡ (μmole)	Relative molar potency§	No. of groups	ED_{50}¶ (mg/kg)	95% confidence limits	Relative molar potency§
 (α-methyldopa)	0·20 2·00 20·00	25 64 82	0·9	1	10	4·7	3·73, 5·92	1
$CH_3(CH_2)_3CH(NHNH_2)COOH$	0·20	24	0·9	1	4	9·0		0·4
$(CH_3)_2CH-CH(NHNH_2)COOH$	0·20 2·00 20·00	26 89 94	0·5	1·8	4	13·0		0·2
	0·20	55	0·2	4·5	4	22·0		0·2
$(C_2H_5)_2C(NHNH_2)COOH$	0·10 1·00 10·00	20 74 100	0·4	2·5	4	24·0		0·1

* All compounds DL mixtures.
† μMole of inhibitor in the 2·8-ml reaction mixtures.
‡ Estimated amount of inhibitor, μmole in 2·8-ml reaction mixture, required to inhibit the reaction rate 50%. See text for conditions.
§ Potency of DL-α-methyldopa taken as 1.
¶ With 9 or 10 groups of 5 mice per drug, regression lines were calculated by the method of least squares, and ED_{50}'s and 95% confidence limits computed. Regression lines were linear and parallel within the limits of experimental error. With 4 groups of mice per drug, ED_{50}'s were determined graphically and are approximations only.
** Oral ED_{50}, 0·56 mg/kg; 95% confidence limits 0·54, 0·60.

After gassing with nitrogen, equilibration at 37 °C, and addition of substrate, the rate of enzymatic decarboxylation in the absence of inhibitors was constant for approximately 30 min. Routinely, the amount of CO_2 evolved in 15 min was taken as a measure of rate of decarboxylation.

Approximate relative potency figures (Table 1) show HMD, α-hydrazino-3,4-dihydroxyphenylpropionic acid and α-hydrazino-3-hydroxyphenylpropionic acid to have 450 to 1500 times the activity of α-methyldopa as inhibitors of DOPA decarboxylase *in vitro*. The remaining aromatic α-hydrazino acids (α-methyl-α-hydrazinophenyl-, α-methyl-α-hydrazino-(3-methoxy-4-hydroxyphenyl)-, and α-hydrazino-(4-methoxyphenyl)-propionic acids) were 4·5 to 20 times as active, the aliphatic derivatives 1 to 2·5 times as active, as α-methyldopa.

It is interesting to note that, at inhibition of 50 per cent, the ratio of concentrations of pyridoxal phosphate to HMD was 0·2 : 0·0006 (333 : 1); however, the ratio of pyridoxal phosphate to aliphatic hydrazino acids, at 50 per cent inhibition, ranged between 0·2 : 1 and 1 : 1.

Inhibition of L-*DOPA decarboxylase* in vivo

Mouse kidney serotonin. The procedure has been described elsewhere.[6] Briefly, groups of mice which had been dosed intraperitoneally 16 hr earlier with 10 mg of a monoamine oxidase inhibitor (phenylisopropylhydrazine, JB-516)/kg were given, by the same route, 100 mg 5-HTP/kg and various amounts of DOPA decarboxylase inhibitor. Serotonin was determined[7] in kidney homogenates 1 hr later, and the dose of inhibitor that would reduce the accumulation of serotonin in kidneys by one-half (ED_{50}) was calculated.

The data (Table 1) show that, *in vivo*, HMD and its desmethyl derivative are 60 to 80 times as potent as the reference compound, DL-α-methyldopa. It is apparent that, while aliphatic α-hydrazino acids were DOPA decarboxylase inhibitors, they were considerably less potent than the hydroxylated, aromatic α-hydrazino acids. The occurrence of a free hydroxyl group on the benzene ring at position 3, and particularly at both 3 and 4, enhanced potency in inhibiting accumulation of kidney serotonin.

HMD administered orally, 30 min prior to 5-HTP, was about one-sixth as potent in this test as was intraperitoneal HMD administered simultaneously with 5-HTP. Orally, its ED_{50} is 0·56 (95% confidence limits, 0·54, 0·60). Compared with α-methyldopa, the hydrazino compounds (Table 1) were less potent *in vivo* than *in vitro*; however, comparison among the hydrazines themselves shows quite remarkable correlation between relative potency estimates *in vitro* and *in vivo*.

Excretion of serotonin by rats. Male albino rats (Holtzman) weighing about 200 g each, were dosed intraperitoneally with 5 mg of the monoamine oxidase inhibitor, phenylisopropylhydrazine (JB-516)/kg, in order to prevent conversion of serotonin to 5-hydroxyindoleacetic acid. The rats were given single oral doses 16 hr later of from 1 to 81 mg of HMD/kg, and one-half hour later, single intraperitoneal doses of 12 mg 5-HTP/kg. Twenty-four-hour urines were assayed for serotonin by the fluorometric method of Bogdanski *et al.*[7]

The excretion of serotonin by the rats was significantly decreased by the administration of HMD (Table 2). It is estimated that an oral dose of about 1·5 mg of the DOPA decarboxylase inhibitor/kg would have decreased the excretion of serotonin by about 50 per cent.

Decarboxylation of ^{14}C-OOH-*labeled amino acids in the rat.* Male albino rats (Holtzman), weighing 80–120 g each, were housed* in individual all-glass metabolism cages.[8] The animals were given 0 or 50 mg of HMD/kg intraperitoneally, then from 2·4 to 6·8 mg of carboxyl-^{14}C-amino acid/kg by the same route. Expired air was passed through a small bubbler which contained 15 ml of 10% NaOH; the latter was

TABLE 2. INFLUENCE OF ORALLY ADMINISTERED HMD UPON SEROTONIN EXCRETION BY 5-HTP-DOSED RATS

Dose (mg/kg)			Mean 24-hr serotonin excretion (μg/kg)
JB-516*	5-HTP†	HMD	
5	0	0	36 ± 11‡
5	0	81	50 ± 2
5	12	0	1022 ± 229
5	12	1	695 ± 160
5	12	3	289 ± 88
5	12	9	151 ± 32
5	12	27	107 ± 9
5	12	81	102 ± 3

* Phenylisopropylhydrazine, give i.p. 16 hr before other dosing.
† Intraperitoneally, 30 min after HMD.
‡ Standard deviation; 3 animals per treatment. Calculated dose of HMD required to inhibit the excretion of serotonin after administration of 5-HTP, approximately 50%, 1·5 mg/kg.

changed each 30 min for 6 hr. Finally, air expired from 6 to 24 hr was passed through a large gas washing column containing 100 ml NaOH. After 24 hr, carcasses and feces were homogenized in water and decolorized by heating with alkali and hydrogen peroxide.[9] Decolorized homogenates, alkali-trapped CO_2, and diluted urines, 1 ml of each, were added to 20 ml phosphor solution (toluene : ethanol, 1 : 1, containing 4 g PPO, 100 mg POPOP, and 30 g Cab-o-sil per liter) and counted in the Packard Tri-Carb liquid scintillation counter. The high voltage was set at 1000 V; internal standards were run for sets of measurements where the ratio of counts at 10–50 V to counts at 10-∞ V were in a range of ±5 per cent. Counting efficiency was 43–45 per cent.

The three aromatic amino acids studied, L-phenylalanine, L-tyrosine, and L-α-methyldopa (methyldopa) were extensively decarboxylated in the rat (Table 3), and the rate of decarboxylation, particularly during the first few hours after injection (Fig. 1), was sharply decreased by HMD. On the other hand, production *in vivo* of ^{14}C-O_2 from DL-1-^{14}C-glutamic acid was not noticeably influenced by injection of HMD.

After the administration of HMD, a greater proportion of the radioactivity from carboxyl-labeled methyldopa appeared in the feces and carcass than after methyldopa alone. The radioactivity in the carcass could be mostly in unexcreted feces, but direct evidence to prove this point is not available at present.

Retention of both L-phenylalanine and L-tyrosine appeared to be increased in the presence of HMD. In these cases, since fecal radioactivity was not increased, the retained amino acids may have been incorporated into body constituents.

* Delmar Scientific Laboratories, Chicago, Ill.

Brain serotonin. Mice were given two doses of DOPA decarboxylase inhibitor, 100 mg/kg, spaced 16 hr apart; 1 hr after the second dose, brain serotonin was determined according to Bogdanski *et al.*[7] Under these conditions and with much smaller doses, both reversible (harmaline), and irreversible (phenylisopropylhydrazine) amine oxidase inhibitors produce striking increases in brain serotonin.[10, 11] However,

TABLE 3. INFLUENCE OF HMD ON DECARBOXYLATION OF CARBOXYL-^{14}C-AMINO ACIDS IN THE RAT (INTRAPERITONEAL ADMINISTRATION)

Dose†‡	Radioactivity in fraction (% of dose*)				
	CO_2 0–6 hr	CO_2 6–24 hr	Urine	Carcass	Feces
L-Phenylalanine	16·70	4·93	2·93	74·15	1·34
L-Phen. + HMD	4·46	5·58	1·13	87·66	1·08
L-Tyrosine	32·11	9·88	2·26	53·56	2·21
L-Tyr. + HMD	12·71	9·44	1·61	74·76	1·66
L-α-Methyldopa	48·85	2·00	45·60	2·95	0·61
L-α-Meth. + HMD	3·19	0·66	52·76	16·22	27·18
DL-Glutamic acid	43·24	1·99	50·20	3·75	0·82
DL-Glut. + HMD	44·77	2·04	45·65	7·20	0·36

* Average values from 2 rats each treatment.

† L-Phenylalanine, New England Nuclear Corp., 6·5 mc/mmole; dose 10 μc, 0·24 mg/100 g rat. L-Tyrosine, Calif. Corp. for Biochem. Res., 6·8 mc/mmole; dose 10 μc, 0·28 mg/100 g rat. L-α-Methyldopa, Merck Sharp & Dohme Res. Labs., 0·72 mc/mmole; dose 1·7 μc, 0·5 mg/100 g rat. DL-Glutamic acid, Merck Sharp & Dohme, Canada, 4·4 mc/mmole; dose 20 μc, 0·68 mg/100 g rat.

‡ HMD, 5 mg/100 g rat, i.p.

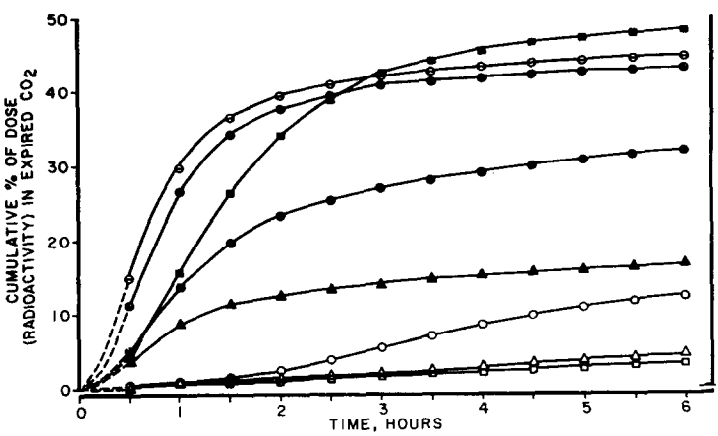


FIG. 1. Rate of decarboxylation of amino acids in rats. Effect of HMD. ▲ L-Phenylalanine. △ L-Phenylalanine + HMD. ● L-Tyrosine. ○ L-Tyrosine + HMD. ■ L-α-Methyldopa. □ L-α-Methyldopa + HMD. ⊕ DL-Glutamic acid. ⊖ DL-Glutamic acid + HMD. Each curve average data from two rats. For doses see Table 3.

a significant rise in brain serotonin concentration was not produced by HMD, α-hydrazino-3,4-dihydroxyphenylpropionic acid, or α-hydrazino-3-hydroxyphenylpropionic acid (Table 4). Obviously, the α-hydrazino acids are not efficient monoamine oxidase inhibitors, at least in the brain *in vivo*. As will be shown later, however, HMD does not efficiently pass the blood–brain barrier.

An effect of HMD upon brain serotonin could be demonstrated in the rat, when the animals also received doses of JB-516 and 5-hydroxytryptophan (Fig. 2), but not in the absence of the latter (Table 5). It is clear that in the rat, as in the mouse, HMD is not an efficient inhibitor of decarboxylation in the central nervous system.

TABLE 4. INFLUENCE OF HMD AND RELATED COMPOUNDS ON BRAIN SEROTONIN IN THE MOUSE

Compound*	Brain serotonin† (μg/g)
None	0·74, 0·81
HMD	0·98, 0·84
α-Hydrazino-3,4-dihydroxyphenylpropionic acid	0·74, 0·80
α-Hydrazino-3-hydroxyphenylpropionic acid	0·78, 0·76

* Dose: 2 × 100 mg/kg, i.p., spaced 16 hr apart. Animals killed 1 hr after second dose.

† Two pools of 5 mice per treatment. Analysis of variance shows no significant differences among groups ($P > 0·05$).

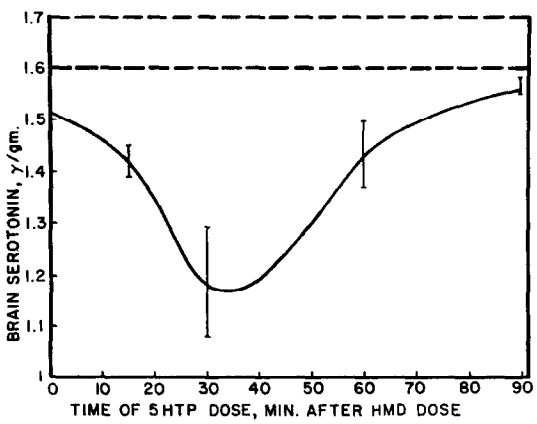


FIG. 2. Effect of HMD on serotonin in brains of 5-HTP-dosed rats. Rats pre-dosed with 5 mg phenylisopropylhydrazine/kg (16 hr); HMD (75 mg/kg) administered at various times prior to 5-HTP (50 mg/kg). Animals killed 15 min after 5-HTP dose. Vertical bars represent range of values from duplicate groups of 3 rats each. Horizontal dotted lines show the range of duplicate control (no HMD) groups.

The following analysis of variance table shows a significant difference for treatment; e.g. for HMD:

Source of error	df	ss	ms	F	P
Total	11	0·3127			
Groups	5	0·2549	0·05098	5·29	<0·05
In groups	6	0·0578	0·00963		

The metabolism and distribution of HMD in the rat. Rats were given single oral or intraperitoneal doses of carboxyl-^{14}C-HMD.* Tissue homogenates and excreta were plated, dried, and counted in a micromil-window flow-gas counter. Appropriate self-absorption corrections were applied. Unmistakable evidence (Table 6) was obtained

* ^{14}C-labeled HMD and methyldopa were kindly supplied by Dr. H. E. Mertel, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.

that the rat decarboxylated the α-hydrazino acid. About 35 per cent of the orally administered compound appeared to be decarboxylated; if this were a specific L-hydrazino acid decarboxylation, some 70 per cent of the L-isomer would be implicated as being decarboxylated. After intraperitoneal administration, only about 9 per cent of the HMD appeared as radioactivity in expired CO_2, and a proportionately larger amount came out in the urine.

TABLE 5. INFLUENCE OF HMD ON SEROTONIN IN THE BRAINS OF RATS

Time after HMD (min)	Serotonin in brain* (μg/g)
Control	1·60, 1·37
15	1·37, 1·34
30	1·50, 1·43
45	1·45, 1·50
75	1·57, 1·38
105	1·36, 1·33

* Rats dosed 16 hr previously with 5 mg phenylisopropylhydrazine/kg i.p. HMD, 75 mg/kg, i.p. Duplicate groups of three rats each time. Analysis of variance shows no significant differences ($P > 0·05$) among treatments.

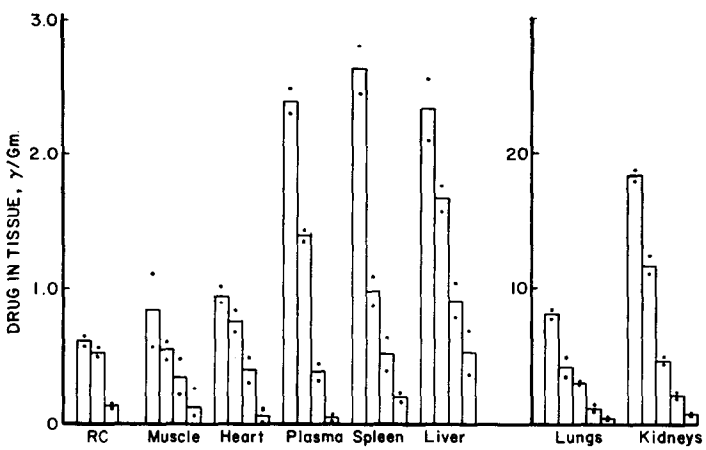


FIG. 3. Distribution of radioactivity in rat tissues after administration of 5 mg carboxyl-^{14}C-HMD/kg. First bar in each group represents data from animals killed 0·5 hr after drug administration; second bar, 1 hr; third bar, 2 hr; fourth bar, 6 hr; fifth bar (when measurable), 24 hr. Each bar represents average data from two animals, the individual values being represented by dots.

The distribution of radioactivity in the tissues of rats after intravenous administration of 1-^{14}C-HMD is shown in Fig. 3. The kidneys, and to a lesser extent the lungs, concentrated the drug (or its decomposition products). Plasma, spleen, and liver contained less radioactivity than kidneys and lungs; red cells, muscle, and heart, still less; and the brain contained too little to measure with certainty.

Radioactivity was rapidly cleared from the tissues. Counts in all tissues in 6 hr had declined to 20 per cent or less of the 30-min values; 24 hr after administration of labeled HMD, only the lungs and kidneys retained measurable radioactivity, corresponding to 0·5–0·7 μg HMD/g.

TABLE 6. METABOLISM OF ^{14}C-OOH-HMD IN RATS

Fraction	Dose in fraction (% ± s.d.)*	
	After p.o. dose	After i.p. dose
Urine	13·5 ± 2·14	84·5 ± 7·24
Carcass	20·5 ± 4·95	4·8 ± 5·50
Feces	27·5 ± 7·77	1·4 ± 1·38
4-hr CO_2	14·8 ± 7·42	3·3 ± 1·26
4 to 24-hr CO_2	20·0 ± 7·44	5·8 ± 2·22

* Average of data from 4 rats each route of administration; dose, 5 mg/kg.

DISCUSSION

α-Methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid is a potent inhibitor *in vitro* of DOPA decarboxylase; *in vivo*, its activity is modified by a number of conditions. First, its activity is easily demonstrated in the kidneys where the drug is concentrated. In the brain, however, very little or no activity is evident because HMD does not enter the brain in measurable quantities. Second, HMD is apparently decarboxylated, the degree of metabolism by this means depending upon the route of administration.

Twenty-four hours after the oral administration of 1-^{14}C-HMD to rats, some 48 per cent of the radioactivity remained in the feces and carcass. Since distribution experiments showed no large accumulation of radioactivity in the tissues after 24 hr, radioactivity in the total carcass probably was present mostly as unabsorbed drug in the gastrointestinal tract. Thus, it appears that about one-half of the oral dose was absorbed; however, decarboxylation of HMD was much more pronounced after oral than after intraperitoneal administration. Slower absorption from the gut and the resulting greater efficiency of metabolism could account for the lack of potency of orally administered HMD (one-sixth of intraperitoneal potency). Alternatively, bacterial decarboxylation of HMD in the gastrointestinal tract may explain, at least in part, the augmented production of ^{14}C-O_2 from orally administered drug. It may be fortuitous, but it is interesting that the ratio of radioactivity in the urine after oral and intraperitoneal dosing of rats with HMD was the same as the ratio of inhibiting potency of DOPA decarboxylase after administration by these two routes; i.e. one-sixth.

By various methods, HMD has been shown to inhibit decarboxylation of L-DOPA, L-phenylalanine, L-tyrosine, L-α-methyldopa, and DL-5-hydroxytryptophan. HMD did not alter decarboxylation of glutamic acid *in vivo*. Since glutamic acid decarboxylase occurs principally in the brain,[12] and since HMD does not have ready access to this organ, no inhibition of glutamic acid decarboxylase would be expected. The principal

metabolic fate of glutamic acid is probably via transamination with oxalacetic acid and decarboxylation of the resulting α-ketoglutaric acid.[13] The failure of HMD to inhibit the metabolic production of ^{14}C-O_2 from carboxyl-labeled glutamic acid suggests that the drug is not a potent carbonyl-trapping agent since compounds of the latter class are known to interfere with glutamic acid transamination.[3, 14, 15]

The three α-hydrazino acids that were the most potent inhibitors of DOPA decarboxylase *in vitro* and *in vivo* are closely related structurally to substrates of the enzyme; i.e. each is a 3-hydroxy- or 3,4-dihydroxyphenylpropionic acid derivative. The less active inhibitors generally are not so closely related to the substrates, and their inhibitory activity may be primarily due to pyridoxal phosphate trapping. It seems reasonable to conclude that HMD inhibits DOPA decarboxylase mainly by virtue of its structural similarity to the substrate. HMD may also trap pyridoxal phosphate to some degree, but this function probably does not contribute notably to its ability to inhibit DOPA decarboxylase. It is worth pointing out that the α-methyl group of HMD apparently does not contribute to enzyme-inhibiting potency.

If HMD were simply decarboxylated in the animal, the product, 3,4-dihydroxyphenylisopropylhydrazine, would be expected to inhibit monoamine oxidase. The fact that there was no evidence for decreased monoamine oxidase activity *in vivo* after the administration of HMD, suggests that if 3,4-dihydroxyphenylisopropylhydrazine was formed, it was rapidly metabolized so that effective concentrations did not accumulate.

The increased excretion of radioactivity in feces by rats which received HMD in addition to carboxyl-^{14}C-α-methyldopa, intraperitoneally, suggests that the liver is capable of excreting, via bile, either the amino acid or an undecarboxylated metabolite. Since over-all recovery of radioactivity administered as α-methyldopa amounted to 95–105 per cent, and no special precautions were taken to prevent loss of CO_2 from feces during drying, homogenizing, and counting, fecal radioactivity probably does not represent ^{14}C-O_2. However, fecal radioactivity could be present, for example, as 3-methoxy-4-hydroxy-α-methylphenylalanine, a metabolite of α-methyldopa in the rat.[16]

Retention in the carcass of radioactivity from injected L-1-^{14}C-phenylalanine and L-1-^{14}C-tyrosine was increased by HMD. It is doubtful that the greater retention reflects incorporation of larger amounts of these aromatic amino acids into protein. More probably, protection of these amino acids from decarboxylation by HMD permitted greater exchange of labeled with cold compounds in the amino acid pool.

It was noted (Table 3) that inhibition of the urinary excretion of serotonin by rats which received 5-HTP (and JB-516) was not complete, even at the highest doses of HMD employed. Possibly, some decarboxylation *in vivo* of 5-HTP occurred independently of DOPA decarboxylase. It appears equally likely particularly since HMD was injected 30 min before 5-HTP, that because of metabolism and excretion HMD concentrations in the tissues declined to suboptimal levels before 5-HTP was entirely cleared from the tissues, and some conversion of the latter to serotonin occurred.

The mechanism by which HMD depressed brain serotonin concentrations in JB-516, 5-HTP rats is not clear. It might be reasoned that prevention of serotonin formation in tissues other than the brain should result in presentation to the brain of more 5-HTP, and consequently an *increase* in brain serotonin. However, serotonin depresses kidney function[17]; HMD prevents formation of serotonin from 5-HTP and

consequently may promote excretion of 5-HTP. As a result, the amount of 5-HTP presented to the brain could actually be *decreased* by HMD. This interpretation must be examined experimentally.

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