Biochemical Pharmacology, Vol. 19, pp. 1601–1614. Pergamon Press. 1970. Printed in Great Britain

SOME BIOCHEMICAL AND PHARMACOLOGICAL ACTIONS OF α-METHYLPHENYLALANINE

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(*Received* 19 *June* 1969; *accepted* 5 *September* 1969)

Abstract—The catecholamine-depleting activity and certain pharmacological actions of *l*-α-methylphenylalanine, a tyrosine hydroxylase inhibitor, were compared in a number of species with those of the related α-methyl amino acids, *l*-α-methyl-*p*-tyrosine and *dl*-α-methyl-*m*-tyrosine. α-Methylphenylalanine was more active than α-methyl-*p*-tyrosine in depleting heart norepinephrine, but was less effective in reducing central amines; in addition, its duration of action in the heart was more prolonged. α-Methyl-*m*-tyrosine proved to be the most active compound on both brain and heart catecholamines. Metaraminol was found to be a metabolite of α-methylphenylalanine and was identified in the hearts, brains and adrenals of mice, rats and dogs. No overt signs of sedation were seen in dogs or squirrel monkeys given α-methylphenylalanine, although loss of avoidance responding could be demonstrated. This contrasted with results obtained with α-methyl-*m*-tyrosine, which produces stimulation and an increase in lever-pressing behavior. α-Methylphenylalanine, as found with α-methyl-*p*-tyrosine and α-methyl-*m*-tyrosine, reduced cardiac responses to the indirect sympathomimetic amines and to adrenergic nerve stimulation. It was concluded that the pattern of biochemical and pharmacologic events after α-methylphenylalanine falls between those because of α-methyl-*p*-tyrosine, a relatively pure tyrosine hydroxylase inhibitor, and α-methyl-*m*-tyrosine, whose effects are related predominantly to its rapid metabolism to metaraminol with a consequent release and depletion of catecholamines.

A NUMBER of α-methyl aromatic amino acids have been demonstrated to interfere with the biosynthesis of the adrenergic transmitters, dopamine and norepinephrine. α-Methyl-3,4-dihydroxyphenylalanine (α-methyldopa) and α-methyl-*meta*-tyrosine were the first of these agents to receive attention because of their ability to inhibit aromatic amino acid decarboxylase.[1] Subsequently it was discovered that except for the relatively short effect on brain serotonin and dopamine levels, the predicted and demonstrated ability of these agents to reduce tissue levels of catecholamines were not associated with their ability to inhibit decarboxylation.[2, 3] It is now generally agreed that the mechanism of their norepinephrine depleting actions is related to the formation of α-methyl amines which replace norepinephrine in the adrenergic neuron, presumably by a simple displacement, and thereby become substitute adrenergic transmitters.[4] These α-methyl amines are α-methyldopamine and/or α-methylnorepinephrine in the case of methyldopa, and α-methyl-*meta*-tyramine and/or *meta*-hydroxynorephedrine (metaraminol) as derived from α-methyl-*meta*-tyrosine.

Another α-methyl aromatic amino acid which is of interest because of its biochemical activity on catecholamine biosynthesis is α-methyl-*p*-tyrosine. This agent, described by Nagatsu *et al.*[5] as an inhibitor of tyrosine hydroxylase, reduces brain norepinephrine and dopamine more or less in parallel and has relatively much less effect on

peripheral norepinephrine.[6] Compared to α-methyldopa and α-methyl-*meta*-tyrosine, α-methyl-*p*-tyrosine has no effect on brain serotonin levels and its action in reducing catecholamines is shorter lasting. The mechanism of its depleting effects on norepinephrine and dopamine is believed to be related to its ability to inhibit tyrosine hydroxylase, although α-methylnorepinephrine is formed to a small extent.[7]

α-Methylphenylalanine, the subject of the present report, was found by Udenfriend *et al.*[8] to be capable of inhibiting tyrosine hydroxylase *in vitro*. Initial experiments in this laboratory showed that in mice its effects on brain dopamine and norepinephrine resembled those of α-methyl-*p*-tyrosine; however, it produced a substantially greater and longer enduring reduction of heart norepinephrine than did α-methyl-*p*-tyrosine. It was found that the reduction in norepinephrine after α-methylphenylalanine was associated with the appearance of an *o*-phthalaldehyde-reactive material, subsequently identified as metaraminol. Earlier studies had shown that metaraminol was a potent displacer of norepinephrine.[9, 10] For this reason additional experiments reported herein were undertaken to compare in more detail the activity of α-methylphenylalanine with α-methyl-*p*-tyrosine and to determine whether the metaraminol formed after α-methylphenylalanine might account for some of the observed differences. Some comparisons with α-methyl-*meta*-tyrosine were also made since the latter agent is well known to be metabolized to metaraminol.[11, 12]

Certain of the data given herein have been previously presented.[13–16]

METHODS

Biochemical. Norepinephrine was determined by iodine oxidation either of alumina-purified perchloric acid extracts[17] or of acid extracts of butanol homogenates of tissues.[3, 18] Metaraminol was determined by the method of Shore and Alpers[19] directly in perchloric acid extracts of tissues or in eluates from paper chromatograms of urine. In urine α-methylphenylalanine and α-methyl-*meta*-tyrosine were purified through column chromatography (Amberlite CG-120) and thin-layer chromatography. α-Methyl-*meta*-tyrosine was measured by the method of Shore and Alpers.[19] α-Methylphenylalanine was nitrated, reduced, diazotized and coupled with *N'*-naphthylethylenediamine, essentially according to the method for phenylalanine described by Hess and Sullivan.[20] Other determinations used were as follows: serotonin, Bogdanski *et al.*;[21] α-methyltyramine and α-methyltyrosine, Waalkes and Udenfriend;[22] α-methylnorepinephrine, Waldeck.[23] Metaraminol was also identified by isotope dilution: ^{14}C metaraminol (^{14}C-α-methyl) was added to perchloric acid extracts of hearts from normal and α-methylphenylalanine-treated rats. The amines were extracted from pH 9 aqueous solutions with 1:1 *n*-butanol–heptane, then back into dilute hydrochloric acid, and further purified by adsorption on a column of Dowex 50, elution with ammonium hydroxide, and chromatography on paper (butanol–acetic acid–water, 4:1:1).

Pharmacological. a. The acute effects of intravenously administered l-α-methylphenylalanine on autonomic function were examined in vinbarbital-anesthetized (50 mg/kg) mongrel dogs by recording pressor and chronotropic responses to intravenous injection of norepinephrine (1 μg/kg), phenethylamine (100 μg/kg), amphetamine (500 μg/kg) and to central vagal stimulation (supramaximal current, 5-msec duration for 10 sec). The procedure employed is identical to that described in detail previously.[24]

At the termination of the experiment, the atria were removed and frozen, and later analyzed for norepinephrine and metaraminol as outlined above.

b. The effects on sympathetic transmission were studied in vinbarbital (50 mg/kg, i.v.) anesthetized, artificially respired dogs by determining the heart rate response to stimulation of the right postganglionic portion of the cardioaccelerator nerve. Maximal stimuli of 3-msec duration were delivered from a Tektronix stimulator through a monopolar electrode for 10 sec at increasing frequencies of 0·5, 1·58, 5·0, 15·8 and 50 cps. Intervals of 5 min were allowed to elapse between stimulation periods to permit establishment of a steady state. At the end of the experiment the atria were removed and frozen and later analyzed for norepinephrine and metaraminol. In these experiments mongrel dogs were pretreated with α-methyl-*meta*-tyrosine, α-methyl-*p*-tyrosine, or reserpine, while studies with α-methylphenylalanine and metaraminol were conducted in beagles.

c. *Behavioral studies.* (1) Squirrel monkeys were trained in an avoidance lever-pressing response as described by Hanson *et al.*[25] During training and testing sessions the animals were seated in test stands but at other periods were housed in individual cages with free access to food and water. The avoidance schedule was programmed with 0·5-sec electric shocks each 36 sec unless a lever response was emitted; each lever response reset the 36-sec timer and started a new timing period. Animals were tested every 2 hr for 30 hr and a maximum of 50 shocks could be received during the 50-min test period. α-Methylphenylalanine was suspended in 1% methocellulose and given orally to groups of five or six animals at doses of 150, 300 or 600 mg/kg; control animals received the vehicle.

(2) Beagle dogs were trained in an avoidance situation similar to that described above with the exception that the dogs were trained to lift a light horizontal bar located just above the head. The animals were trained and tested restrained by a sling which kept the proper relationship between the animal and the test bar. Two dogs were observed for 30 hr using a 30-min test session every 2 hr on a control day and after receiving 100 and 300 mg/kg of α-methylphenylalanine. The cumulative effect of α-methylphenylalanine was studied in three dogs; although two of these animals were also used in the single-dose experiments, several months separated the two studies. Animals were tested continuously daily for 4 hr and after an 8-day control period animals were given α-methylphenylalanine, 100 mg/kg per day for 21 days, immediately before the test session. After a 30-day interval without treatment, animals were given α-methyl-*meta*-tyrosine (50 mg/kg, orally) and tested in a similar manner.

Materials. The following compounds used in these experiments were prepared in these laboratories and given as mg base weight per kg: *d*- and *l*-α-methylphenylalanine, *l*-α-methyl-*p*-tyrosine, *l*- and *dl*-α-methyl-*meta*-tyrosine, *l*-metaraminol-*d*-bitartrate, *dl*-α-hydrazino-α-methyldopa and *dl*-α-methyl-*meta*-tyramine.

RESULTS

Catecholamine depletion. The catecholamine-depleting action of the *l*-isomers of α-methylphenylalanine, α-methyltyrosine and α-methyl-*meta*-tyrosine in the brains and hearts of mice 4–6 hr after the intraperitoneal administration of the amino acids is shown in Table 1. At this interval α-methylphenylalanine was ten times more active than α-methyltyrosine in reducing heart norepinephrine but was three times less

TABLE 1. CATECHOLAMINE DEPLETION IN THE MOUSE 4–6 hr AFTER INTRAPERITONEAL ADMINISTRATION OF *l*-α-METHYL AMINO ACIDS*

Dose (mg/kg)	Heart Norepinephrine (μg/g)	Heart Norepinephrine (% Reduction)	Brain Norepinephrine (μg/g)	Brain Norepinephrine (% Reduction)	Brain Dopamine (μg/g)	Brain Dopamine (% Reduction)
l-α-Methylphenylalanine						
0·0	0·81	0	0·47	0	0·60	0
18·75	0·46	44	0·42	11	0·54	7
37·5	0·38	53	0·34	28	0·42	29
75·0	0·28	65	0·28	40	0·34	43
150·0	0·27	67	0·23	51	0·26	57
ED_{50} mg/kg	28·5		131·2		105·0	
95% C. L.	19·9, 40·9		117·5, 146·6		86·3, 127·6	
l-α-Methyl-tyrosine						
0·0	1·10	0	0·54	0	0·75	0
15·0			0·37	32	0·54	28
45·0			0·27	50	0·39	48
100·0	0·76	31				
135·0			0·16	70	0·18	76
200·0	0·62	44				
ED_{50} mg/kg		*ca.* 280	42·9		44·3	
95% C. L.			39·8, 46·1		36·1, 54·3	
l-α-Methyl-*meta*-tyrosine						
0·0	0·84	0	0·48	0	0·67	
1·0	0·48	43	0·41	15	0·67	0
3·2	0·28	67	0·28	42	0·60	10
10·0	0·16	81	0·15	69	0·37	45
ED_{50} mg/kg	1·4		4·5		12·5	
95% C. L.	1·0, 1·9		3·7, 5·5		10·9, 14·3	

* Three groups of five mice per dose level. ED_{50} and confidence limits (C. L.) calculated by the method of least squares.

active in depleting brain amines. α-Methyl-*meta*-tyrosine was the most active agent in reducing amines in both the brain and heart.

The time course of amine depletion in the brain and heart was studied in mice after the intraperitoneal administration of α-methylphenylalanine (100 mg/kg), α-methyltyrosine (75 mg/kg), and α-methyl-*meta*-tyrosine (3·5 mg/kg). Four hours after treatment there was roughly equivalent depression of brain norepinephrine by all 3 compounds (Table 2). Brain dopamine was affected to a greater extent by α-methyltyrosine than by α-methylphenylalanine and was reduced transiently and to a minor degree by the dose of α-methyl-*meta*-tyrosine used in these studies. Sixteen hours after the administration of the inhibitor both dopamine and norepinephrine concentrations had returned almost to normal in the α-methylphenylalanine-treated animals. Reduction of norepinephrine in the α-methyl-*meta*-tyrosine group was similar to that obtained at 1 and 4 hr while for α-methyltyrosine the reduction in the concentration of central catecholamines was significantly less than in the 4-hr group. Neither α-methylphenylalanine nor α-methyltyrosine altered brain serotonin, although this amine was transiently reduced by α-methyl-*meta*-tyrosine, possibly a reflection of its moderate ability to inhibit decarboxylation.

In the heart both α-methylphenylalanine (100 mg/kg) and α-methyl-*meta*-tyrosine (3·5 mg/kg) produced a 60–70 per cent reduction in the concentration of norepinephrine; this effect persisted for 16 hr (Table 2). However, after α-methyltyrosine (75 mg/kg), and in contrast to its activity in the brain, the reduction of heart norepinephrine was minimal. In the mouse the *d*-isomer of α-methylphenylalanine was not

TABLE 2. TIME COURSE OF AMINE DEPLETION IN THE MOUSE AFTER INTRAPERITONEAL ADMINISTRATION OF THE *l*-α-METHYL AMINO ACIDS

Time (hr)	Heart Norepinephrine (μg/g)	Heart Norepinephrine (% Reduction)	Brain Norepinephrine (μg/g)	Brain Norepinephrine (% Reduction)	Brain Dopamine (μg/g)	Brain Dopamine (% Reduction)
l-α-Methylphenylalanine, 100 mg/kg						
0	0·71	0	0·46	0	0·64	0
1	0·54	23	0·37	18		
4	0·27	62	0·25	45	0·44	31
16	0·27	62	0·42	9	0·65	0
l-α-Methyltyrosine, 75 mg/kg						
0	1·07	0	0·54	0	0·62	0
1	0·96	11	0·44	21	0·47	24
4	0·94	13	0·29	47	0·24	61
16	0·96	11	0·43	22	0·53	15
α-Methyl-*meta*-tyrosine, 3·5 mg/kg*						
0	0·88	0	0·46	0	0·64	0
1	0·48	45	0·32	30	0·57	11
4	0·35	60	0·31	33	0·59	8
16	0·28	68	0·33	28	0·64	0

* Given as 7 mg/kg of *dl* mixture.

effective in lowering brain catecholamines or heart norepinephrine nor did it interfere with the depleting action of the *l*-isomer (unpublished observation).

α-Methylphenylalanine is well absorbed orally as shown by the similar norepinephrine-depleting activity in the mouse heart 16 hr after administration of the amino acid, wherein the ED_{50} after oral administration was 15·1 mg/kg [95 per cent confidence limits (C. L.) 12·2, 18·6] and after intraperitoneal administration 22·4 mg/kg (95 per cent C. L. 19·8, 25·4).

The prolonged norepinephrine-depleting actions after α-methyl-*meta*-tyrosine have been shown to be because of the metabolic conversion of the amino acid to metaraminol. For this reason tissues of α-methylphenylalanine-treated mice were examined for the presence of metaraminol to ascertain if the catecholamine-depleting action after α-methylphenylalanine was also attributable to its metabolic conversion to metaraminol. Metaraminol was detected by the *o*-phthalaldehyde reaction in the hearts but not the brains of mice given a single 100 mg/kg oral dose of α-methylphenylalanine. The metaraminol concentration in the heart was about one-half of the total amine concentration required to account for the missing norepinephrine on a mole-for-mole basis. (Perchloric acid tissue extracts were used.) Additional evidence that the *o*-phthalaldehyde reactive substance was metaraminol was obtained by isotope dilution. ^{14}C metaraminol was added to heart tissue from treated and control animals. The tissue was homogenized in perchloric acid, and the amines were purified as described in the experimental section. Material with R_f 0·67 was eluted, counted and subjected to the *o*-phthalaldehyde reaction. The results demonstrated that the hearts from the α-methylphenylalanine-treated animals contained 0·18 μg/g of metaraminol.

Although metaraminol was not detected in the brain of mice given a single 10 mg/kg oral dose of α-methylphenylalanine, the amine metabolite was present in the brain of animals 16 hr after administration of 300 mg/kg in one or four daily doses. Under these conditions the concentration of metaraminol was sufficient to account approximately for the norepinephrine deficit (Table 3).

TABLE 3. NOREPINEPHRINE AND METARAMINOL CONCENTRATION IN MOUSE HEART AND BRAIN AFTER MULTIPLE TREATMENT WITH *l*-α-METHYLPHENYLALANINE*

Number of doses	Concentration of amines in the heart			
	Norepinephrine		Metaraminol	
	(μg/g)	(% Decrease)	(μg/g)	(% Normal norepi.)
0	0·57		0	
1	0·10	−82	0·23	41
4	0·07	−89	0·26	46
	Concentration of amines in the brain			
0	0·35		0	
1	0·28	−20	0·09	26
4	0·24	−32	0·09	26

* Three hundred mg per kg, p.o., per day; assay 16 hr after final dose; three groups of five mice per treatment.

TABLE 4. EFFECT OF *l*-α-METHYLPHENYLALANINE UPON TISSUE NOREPINEPHRINE CONCENTRATION IN RATS AFTER INTRAPERITONEAL ADMINISTRATION*

Dose (mg/kg)	Tissue amine (μg/g)					
	Atria		Ventricles		Brain	
	Norepi.	Metaraminol	Norepi.	Metaraminol	Norepi.	Metaraminol
0	2·19	0·00	0·67	0·00	0·39	0·00
33	1·54	0·18	0·47	0·08	0·36	0·00
100	1·40	0·26	0·46	0·18	0·32	0·01
300	1·29	0·24	0·37	0·30	0·25	0·07

* Three groups of six rats per dose; animals were sacrificed 16 hr after administration of α-methylphenylalanine.

The effects of α-methylphenylalanine were also examined in the rat and dog and were compared to the data obtained in the mouse. As may be seen in Table 4, in the rat α-methylphenylalanine was less effective in reducing brain and heart norepinephrine, and relatively large single doses were required to demonstrate depletion. Also, as in the mouse, the hearts contained metaraminol.

Dogs, too, required relatively large doses of α-methylphenylalanine to effect depletion of norepinephrine. In dogs, 16 hr after administration of a single 300 mg/kg oral dose of α-methylphenylalanine, there was a 50 per cent reduction in the concentration of norepinephrine in both the atria (2·42 to 1·18 μg/g) and brain stem (0·52 to 0·30 μg/g) (Table 5). As may be seen, the effects of repeated doses were cumulative to some extent since 50 per cent reduction of tissue amine was achieved also by daily treatment for 3 days with 33 and 100 mg/kg of the amino acid; after 7 days of treatment there were further, although smaller, reductions in tissue norepinephrine concentrations. A reciprocal relationship was observed between the concentrations of metaraminol and norepinephrine, and, as in the mouse and rat, norepinephrine depletion generally was greater than would result from a mole-for-mole displacement of the catecholamine by metaraminol. In the dog brain, dopamine and serotonin concentrations as well as adrenal catecholamines were not significantly affected by α-methylphenylalanine; however, the adrenals did contain low concentrations of metaraminol ranging from 0·3 to 5 μg/g of tissue (Table 5).

TABLE 5. TISSUE AMINES IN BEAGLE DOGS GIVEN *l*-α-METHYLPHENYLALANINE ORALLY FOR 1, 3 AND 7 days*

	Atrial amine (μg/g)						Ventricular amine (μg/g)					
Dose	Norepinephrine			Metaraminol			Norepinephrine			Metaraminol		
(mg/kg/day)	1	3	7	1	3	7	1	3	7	1	3	7
0		2·42			0·00			1·06			0·00	
3	1·64	2·12	2·53	0·05	0·06	0·19	1·00	0·88	0·96	0·01	0·01	0·05
11	1·86	1·58	2·16	0·10	0·16	0·42	0·77	0·68	0·87	0·02	0·06	0·11
33	1·62	0·85	1·13	0·16	0·27	0·66	0·64	0·58	0·80	0·02	0·07	0·21
100	1·43	0·87	0·54	0·37	0·45	0·49	0·55	0·40	0·35	0·13	0·15	0·18
300	1·18	0·71		0·31	0·54		0·60	0·36		0·17	0·21	
S. D.		0·80			0·11			0·21			0·05	

	Brain stem amine (μg/g)										
Dose	Norepinephrine			Metaraminol			Dopamine			Serotonin	
(mg/kg/day)	1	3	7	1	3	7	1	3	7	3	7
0		0·52			0·00			0·26			0·90
3	0·41	0·44	0·41	0·00	0·03	0·06	0·24	0·16	0·17	0·96	0·85
11	0·43	0·43	0·31	0·00	0·07	0·12	0·19	0·17	0·16	0·86	0·72
33	0·33	0·34	0·18	0·01	0·11	0·19	0·16	0·18	0·15	0·95	0·68
100	0·26	0·22	0·16	0·05	0·14	0·26	0·10	0·07	0·15	0·90	0·60
300	0·30	0·16		0·08	0·20		0·13	0·05		0·59	
S. D.		0·06			0·05			0·12			0·09

	Adrenal amine (μg/g)					
Dose	Epinephrine		Norepinephrine		Metaraminol	
(mg/kg/day)	3	7	3	7	3	7
0	1082	523	217		0·00	
3	1172	572	304	222	0·29	0·79
11	1041	646	159	281	0·57	1·78
33	1038	635	262	225	2·12	2·73
100	1027	644	204	214	2·03	4·32
300	887		206		4·86	
S. D.	90	314	46		1·13	

* Values represent the average obtained in determinations with four animals per treatment except those for the adrenal, serotonin, and the 1-day brain stem are from two animals per treatment. S. D. = Standard Deviation.

Urinary excretion of α-methylphenylalanine by the dog was measured after oral administration of 3·3 and 300 mg/kg. About 60–70 per cent of the administered amino acid was excreted in 24 hr; a small amount (0·01–0·10 per cent) was excreted as α-methyl-*meta*-tyrosine.

Interference with autonomic responses. To determine if the administration of α-methylphenylalanine caused any alteration in autonomic responses or responses to sympathetic nerve stimulation, the following experiments were performed in anesthetized dogs. α-Methylphenylalanine was given in a single i.v. dose of 100 mg/kg, and 16–24 hr later the pressor and chronotropic responses to injected norepinephrine, phenethylamine or amphetamine, or to central vagal stimulation were not different from responses in control animals (Table 6). However, in these animals the concentration of norepinephrine in the atria was reduced to about one-half of normal concentration, and metaraminol was present. In dogs that received oral doses of 100 mg/kg twice daily for 2 days and an additional 100 mg/kg dose 4 hr before the experi-

TABLE 6. PRESSOR AND CHRONOTROPIC RESPONSES TO AUTONOMIC AGENTS IN DOGS PRETREATED WITH *l*-α-METHYLPHENYLALANINE (MEAN ± S.D.)

Treatment (No. animals)*		Norepinephrine		Phenethylamine		Central vagal stim		Amphetamine		Heart (atria)	
		Initial	Increase	Initial	Increase	Initial	Increase	Initial	Increase	Norepi. (μg/g)	Metaraminol (μg/g)
Control											
(15)	B. P.†	142 ± 20	85 ± 16	134 ± 16	126 ± 19	130 ± 18	82 ± 39	132 ± 21	122 ± 39	3·63 ± 1·01	0·0
	H. R.	145 ± 21	43 ± 21	149 ± 20	100 ± 16	152 ± 18	40 ± 27	147 ± 19	98 ± 16		
α-Methylphenylalanine, 100 mg/kg, i.v.											
(4)	B. P.	142 ± 36	91 ± 17	131 ± 29	115 ± 21	130 ± 36	97 ± 16	123 ± 61	98 ± 12	2·00 ± 0·5‡	0·48 ± 0·05
	H. R.	140 ± 23	66 ± 7	139 ± 20	102 ± 21	142 ± 21	61 ± 11	137 ± 21	89 ± 23		
α-Methylphenylalanine, 500 mg/kg total dose§											
(6)	B. P.	119 ± 12	97 ± 10	107 ± 14	80 ± 26‡	112 ± 15	77 ± 29	112 ± 15	55 ± 25‡	0·61 ± 0·46‡	0·72 ± 0·36
	H. R.	138 ± 5	64 ± 21	145 ± 21	60 ± 30‡	141 ± 15	39 ± 38	140 ± 26	71 ± 51		

* Number of animals given each treatment shown in parentheses.
† B. P. = Mean arterial pressure (mm. Hg); H. R. = heart rate per min.
‡ Significantly different from control, P = < 0·05.
§ Given in divided doses of 100 mg per kg, p.o. bid for 2 days, and again 4 hr prior to the acute experiment.

ment, the responses to the indirect-acting sympathomimetic agents phenethylamine and amphetamine were significantly reduced while responses to norepinephrine and to central vagal stimulation were unaltered. Atrial norepinephrine was reduced to 17 per cent of the normal concentration, and somewhat more metaraminol was present than in the tissues from animals that received a single dose of the amino acid.

Interference with sympathetic nerve transmission. Sixteen to 24 hr after the administration of α-methylphenylalanine or metaraminol in doses that produced 90 per cent

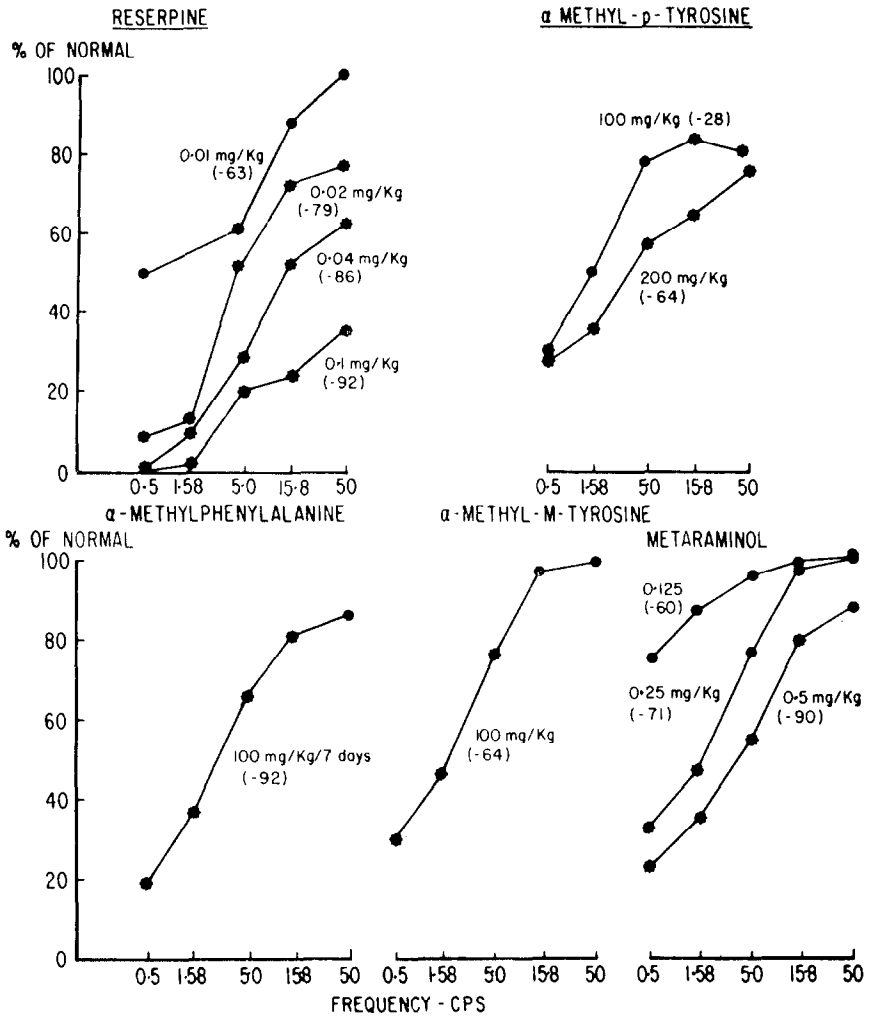

FIG. 1. Chronotropic responses to cardioaccelerator nerve stimulation in the dog. Compounds were given 16–24 hr prior to test as follows: Reserpine — 0·01, 0·02, 0·04 0·1 mg/kg i.v., four animals per dose; l-α-methyltyrosine — 100 mg/kg i.v. 24 hr, four animals and 200 mg/kg given in divided doses of 100 mg/kg 24 and 16 hr, three animals; α-methyl-*meta*-tyrosine given as the *dl* form 200 mg/kg, i.v., four animals; *l*-α-methylphenylalanine, 300 mg/kg p.o. daily for 7 days with the last dose 20–24 hr prior to test, four animals; *l*-metaraminol, 0·125, 0·25, 0·5 mg/kg, p.o., four animals per dose. Per cent depletion of norepinephrine given in parenthesis. * = values significantly different from control ($P = < 0·05$).

depletion of atrial norepinephrine, heart rate responses to low frequency stimulation of the postganglionic portion of the cardioaccelerator nerve were reduced, but the response to 50-cps stimulation was unaffected (Fig. 1). α-Methyltyrosine, 200 mg/kg, given in divided doses of 100 mg/kg, 20 and 16 hr prior to the experiment, reduced atrial norepinephrine only 64 per cent, but significantly reduced the responses to all frequencies of stimulation; although the degree of reduction was less at the higher frequencies, α-methyl-*meta*-tyrosine, in an equieffective depleting dose, reduced the response to the lower frequencies only, thus resembling α-methylphenylalanine and metaraminol. Reserpine at 0·02 mg/kg and α-methyltyrosine at 200 mg/kg significantly reduced responses to all frequencies of stimulation.

Behavioral studies. α-Methylphenylalanine produced a loss of avoidance response in squirrel monkeys trained in an avoidance lever-pressing response and tested for a 50-min session every 2 hr. Oral doses of 150 and 300 mg/kg produced this effect in each of three of six animals and in five of five animals given 600 mg/kg; there was no change in the avoidance pattern, i.e. the number of shocks received in 50 min, in five control animals (Table 7). This effect of α-methylphenylalanine was greatest

TABLE 7. EFFECTS OF *l*-α-METHYLPHENYLALANINE ON SQUIRREL MONKEY AVOIDANCE BEHAVIOR

		α-Methylphenylalanine*		
	Control (N = 5)†	150 mg/kg (N = 6)†	300 mg/kg (N = 6)†	600 mg/kg (N = 5)†
Hours after treatment	Shocks/50 min	Shocks/50 min	Shocks/50 min	Shocks/50 min
0	1·4	1·7	2·7	1·4
2	1·6	3·3	3·5	5·8
4	1·0	8·5	1·7	9·4
6	2·4	8·3	3·0	20·6
8	0·8	5·0	25·8	53·2
10	1·0	21·1	23·1	64·0
12	1·0	42·0	24·5	57·4
14	0·6	34·3	26·3	58·4
16	1·2	32·1	9·3	63·0
18	1·2	30·0	4·5	71·2
20	1·8	18·6	3·3	57·2
22	2·2	16·0	3·7	52·6
24	1·8	6·7	2·7	22·4
26	0·8	4·0	4·2	29·6
28	1·4	3·0	1·3	33·0
30	1·0	3·3	2·7	8·2

* α-Methylphenylalanine given p.o.
† N = Number of animals per group.

10–14 hr after administration of the dose. At no time during the 30-hr test period were signs indicative of central nervous system depression or stimulation evident when the animals were observed in their cages during the time between testing sessions.

Two beagle dogs were tested in a manner similar to the squirrel monkeys; during control studies the animals responded stably and rarely received more than one shock out of a possible fifty per test session. Nearly complete loss of avoidance response was obtained after oral administration of 300 mg/kg of α-methylphenylalanine beginning 4–6 hr after treatment, and was detectable for 8–12 hr. Normal responses were recorded during the remaining testing period. Neither of these animals showed a loss

of avoidance pattern when given an oral dose of 100 mg/kg of α-methylphenylalanine.

The possible cumulation of the effect of α-methylphenylalanine was examined in three dogs given an oral dose of 100 mg/kg daily for 21 consecutive days, and tested for 4 hr beginning immediately after treatment. All animals showed a slight increase in shock incidence during the last quarter of the test session; however, this effect did not increase in magnitude with continued treatment. The average shock incidence per treatment session relative to control sessions (taken for eight consecutive days prior to treatment) was as follows: Dog No. 1, 33/4; dog No. 2, 8·86/2·75; dog No. 3, 3·62/1·25 shocks per session. Under conditions of complete loss of avoidance, 400 shocks could be delivered per session. Thus, in contrast to the marked effect of a single 300 mg/kg dose of α-methylphenylalanine on the avoidance response, the oral administration of 100 mg/kg daily for 21 consecutive days produced a minimal reduction in avoidance behavior with no apparent cumulative effect.

A single oral dose of 50 mg/kg of α-methyl-*meta*-tyrosine caused stimulation as indicated by an increase in the lever press rate; this effect of α-methyl-*meta*-tyrosine was greatest 6–8 hr after treatment. One of the animals showed loss of avoidance after the period of stimulation; animals were essentially normal 24 hr after the treatment.

DISCUSSION

The ability of inhibitors of tyrosine hydroxylation to block the conversion of tyrosine to dopa is manifested *in vivo* by a decrease in norepinephrine content of adrenergically innervated tissues.[26] Both α-methyltyrosine[5] and α-methylphenylalanine[8] are inhibitors of tyrosine hydroxylation but differ in the pattern and duration of their depleting action. The effects of α-methyltyrosine in the mouse, rat and dog were reported to be greater in the brain than the heart, but the effects were also found to be of relatively short duration. A similar pattern of depletion has been observed in the guinea pig.[27] By contrast, as shown by the results reported in this communication, the norepinephrine-depleting effects of α-methylphenylalanine are greater in peripheral tissue (heart) than in the brain and are of longer duration than would be expected if the decrease in norepinephrine were entirely because of inhibition of tyrosine hydroxylation. As demonstrated in the present study, the prolonged depleting action of α-methylphenylalanine can be attributed to the metabolic conversion of the amino acid to metaraminol which is a potent norepinephrine-depleting agent.[11, 12] These findings are similar to those reported for α-methyldopa and α-methyl-*meta*-tyrosine, wherein their depleting action far surpasses their ability to inhibit decarboxylation of dopa to dopamine.[2] The persistent depleting action of α-methyldopa has been shown to be related to its metabolic conversion to α-methylnorepinephrine while that of α-methyl-*meta*-tyrosine is due to its conversion to metaraminol.[10–12] Although α-methyltyrosine has been shown to be metabolized to α-methylnorepinephrine, the quantity of the amine formed was too small to account for the depleting action of this agent.[6]

Metaraminol was identified in the hearts of mice after single doses of less than 25 mg/kg of α-methylphenylalanine; higher doses were required to demonstrate a significant increase in metaraminol in the brain. The rat was less sensitive to the depleting action of α-methylphenylalanine, although the presence of metaraminol in the tissues indicated that this species is able to decarboxylate the amino acid and form the β-hydroxylated metabolite. In the dog there was a dose- and time-dependent

relationship between the decrease in norepinephrine and the accumulation of metaraminol in both the heart and brain. Although adrenal catecholamines were not significantly decreased, metaraminol was identified in this tissue as well. Metaraminol has been reported to replace norepinephrine in adrenergic innervated tissues on a mole-for-mole basis,[28] however, in the studies reported herein, metaraminol did not completely account for the norepinephrine deficit in the heart or brain. That the deficit between norepinephrine depletion and tissue content of metaraminol is not entirely because of the sensitivity of the methods employed was reported in studies with ^{14}C metaraminol,[29] which demonstrated that the time of tissue analysis after treatment was important and, in addition, the difference could not be accounted for by the presence of α-methylnorepinephrine (unpublished observation). However, α-methylphenylalanine is also a tyrosine hydroxylase inhibitor and it is impossible to determine, on the basis of these data, the magnitude of norepinephrine depletion resulting from the two functions of the amino acid.

In dogs about 60–70 per cent of an orally administered dose of α-methylphenylalanine was excreted in the urine in 24 hr, while some of the amino acid was metabolized and excreted as α-methyl-*meta*-tyrosine (0·01–0·10 per cent). Metaraminol was not detected in the urine of the α-methylphenylalanine-treated animals; however, in metaraminol-treated animals 50–75 per cent of the administered dose of the amine is excreted in the urine (unpublished observations). α-Methylphenylalanine was free of significant amounts of *m*-hydroxylated contaminants (i.e. *o*-phthalaldehyde reactive substances). Therefore, it is clear that some α-methylphenylalanine was metabolized to α-methyl-*meta*-tyrosine and metaraminol. Based upon relative potencies in both dogs and mice, 3·5–5 per cent conversion to α-methyl-*meta*-tyrosine would account for the catecholamine-depleting activity of α-methylphenylalanine.

The pharmacological and physiological consequences of the catecholamine reduction effected by α-methylphenylalanine are somewhat similar to those which have been described for methyldopa,[24] α-methyl-*meta*-tyrosine,[24] and metaraminol, *per se*.[30] These agents were found to reduce the cardiovascular response in the dog to the indirect acting amines, amphetamine and phenethylamine, without affecting the response to injected norepinephrine. The response elicited by stimulation of the central end of the vagus nerve was resistant to reduction by methyldopa and α-methyl-*meta*-tyrosine[24] and, as shown in the present work, this also proved to be true for α-methylphenylalanine. This finding indicates that responses requiring release of norepinephrine by nerve impulses are somewhat more resistant to reduction than those related to release by indirect acting sympathomimetic amines. Nonetheless, as shown here for α-methylphenylalanine and α-methyl-*meta*-tyrosine, and elsewhere for methyldopa,[31] heart rate responses induced by stimulation of the accelerans nerve in the dog can be reduced, particularly at low frequency stimulation. It seems probable, in view of the frequency-dependent nature of the reduction, that the vascular response to stimulation of the central end of the vagus nerve is mediated by relatively high frequency discharge rates; hence, the apparent resistance to blockade.

α-Methylphenylalanine produced loss of avoidance behavior in squirrel monkeys and dogs when given in relatively large oral doses; chronic administration of a minimally effective dose to dogs produced no evidence for a cumulation of the effect. The loss of avoidance response after α-methylphenylalanine[15] is qualitatively similar to that observed after α-methyltyrosine;[32] however, unlike α-methyltyrosine, there

were no overt signs of depression or sedation in the animals treated with α-methylphenylalanine. Stimulation after α-methyl-*meta*-tyrosine has been observed in dogs,[24] mice[33] and rats.[34, 35] α-Methyl-*meta*-tyrosine is metabolically converted to metaraminol, and its stimulating effect is attributed to subsequent release of brain amines. α-Methylphenylalanine is also converted to metaraminol but its effect on avoidance behavior is most probably related to its ability to inhibit synthesis of norepinephrine; presumably, its rate of metabolism to metaraminol is too slow to produce overt signs of stimulation such as those observed following α-methyl-*meta*-tyrosine administration.

Acknowledgements—These data were obtained with the technical assistance of G. J. Anderson, E. H. Campbell, C. T. Ludden, M. McGready, J. Stavorski, J. Strouse, J. A. Totaro, H. C. Wenger and J. Witoslawski. We are indebted to H. T. Meriwether and C. Rosenblum for the ^{14}C metaraminol.

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