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THE EFFECT OF THE OPTICAL ISOMERS OF α-METHYL-*p*-TYROSINE UPON BRAIN AND HEART CATECHOLAMINES IN THE MOUSE

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Abstract—*l*-α-Methyl-*p*-tyrosine, an inhibitor of the enzymatic conversion of tyrosine to 3-hydroxytyrosine (DOPA), when injected into mice caused a fall in brain and heart catecholamine concentrations. In the brain, 3,4-dihydroxy-phenethylamine concentrations decreased more rapidly and reached lower levels than did norepinephrine concentrations; norepinephrine was not as readily affected in the heart as in the brain. *d*-α-Methyl-*p*-tyrosine, while having no effect on tissue catecholamine concentrations, potentiated not only the *l*-isomer but also certain catecholamine-depleting agents: e.g. methyldopa, *l*-α-methyl-*m*-tyrosine and 6-hydroxydopamine. However, it did not potentiate metaraminol, reserpine, or guanethidine. This activity of the *d*-isomer is correlated with its ability to promote the accumulation of active compounds in the tissues and is probably related to an effect upon membrane permeability.

α-METHYL-*p*-TYROSINE (AMT) has been shown to inhibit the conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) both *in vitro*[1] and *in vivo*.[2]

Studies with AMT in mice revealed that, while the *d*-isomer was entirely without effect upon tissue catecholamine concentrations, the racemate was more potent than would be predicted from its composition as a 1:1 mixture of active and inactive forms. Further experimentation has demonstrated that *d*-AMT can potentiate the effectiveness not only of *l*-AMT but also of certain catecholamine-depleting agents (e.g. methyldopa, 6-hydroxydopamine) in decreasing tissue catecholamine concentrations. The fact that greater concentrations of the effective agents occurred in the tissues of animals which had also been given *d*-AMT provides a reasonable explanation for the observed potentiation.

EXPERIMENTAL

Female albino mice (Carworth Farms, CF_1) weighing 18–22 g were used. Compounds were injected intraperitoneally in aqueous solution, minimal amounts of hydrochloric acid being used to solubilize AMT. Dosing was on a body weight basis, milligram base per kilogram.

Tissue catecholamines were determined by a modification of the trihydroxy indole method[3] as modified by Hogans (see Porter *et al.*[4]).

Prior to radioactive counting, tissues were dissolved in 1 N KOH and decolorized by the addition of hydrogen peroxide and heating.[5] Aliquots (1 to 2 ml) of the alkaline solutions were added to 20 ml dioxane solution (100 g naphthalene, 7 g PPO, 0·3 g POPOP, 1 liter *p*-dioxane[6]) for liquid scintillation counting. Counting efficiencies, determined by the addition of internal standards, were from 25% to 50% for ^{14}C

and about 5% for ^{3}H. In all cases a sufficient number of counts was accumulated to provide counting reliability of $\pm 3\%$ or better.

For the determination of α-methyltyrosine the tyrosine method of Waalkes and Udenfriend[7] was used, after removal of tyrosine as follows: pools of tissues (2 g brain, 0·4 g heart) were homogenized and made up to 10 ml with 6% trichloroacetic acid. To 6 ml of the clear filtrate were added 0·6 ml pure pyridine and 3 ml 1% ninhydrin. The solution was heated in a 100° bath for 10 min, then cooled quickly.* The solution was acidified (0·7 ml conc. HCl) and washed once with an equal volume of ethyl acetate. Eight ml of the washed solution was run into a column of Dowex-50 (4 g wet resin), buffered at pH 6·5 (0·1 M sodium phosphate buffer). The column was washed with 15 ml water, then 15 ml 3 N HCl. The AMT was eluted with 20 ml of 3 N HCl, and the solution was taken to dryness under reduced pressure; the residue was dissolved in water, and the nitrosonaphthol reaction was applied to the solution. Appropriate recovery and internal standards were processed in a similar way to allow calculation of AMT concentrations in the tissues.

RESULTS

After the injection of *l*-AMT, 75 mg/kg, brain and heart catecholamine concentrations decreased with time, reaching minimal values in about 8 hr (Fig. 1); and at this time, brain catecholamine concentrations (30–40% of normal) were considerably lower than the concentration of norepinephrine in the hearts (75% of normal). In the period between 8 and 10 hr and 32 and 64hr after drug administration, tissue catecholamines returned to normal levels. Although *d*-AMT did not decrease tissue catecholamine concentrations, the administration of 50 mg of the racemate per kg resulted in decreases of the same order of magnitude as those following administration of the *l*-isomer, 75 mg/kg. Peak effects were delayed, however, with the result that in the period 16 to 32 hr the racemate was considerably more effective than the optically active form (Fig. 1).

The 1:1 mixture of isomers as occurs in the racemate was not optimal when catecholamines were measured 16 hr after injection of the compounds. With a constant amount of *l*-isomer (18·8 mg/kg), a ratio of *d*- to *l*-forms in the range of 2:1 to 4:1 was more effective than the racemate (Table 1).

d-AMT but not the *l*-isomer potentiated the effect of methyldopa on tissue norepinephrine (Figs. 2 and 3); ED_{50}'s (brain and heart norepinephrine) for methyldopa were decreased roughly 50% by the administration of *d*-AMT, 30 mg/kg. The potencies of 6-hydroxydopamine and of *l*-α-methyl-*m*-tyrosine were likewise increased by *d*-AMT (Table 2). However, the compound (30 mg/kg) did not influence the depletion of heart norepinephrine ($P > 0{\cdot}25$) after the administration of metaraminol (0·08 mg/kg), reserpine (0·05 mg/kg), or guanethidine (4 mg/kg) at doses that cause about 50% depletion of catecholamine.

Radioactivity in tissues measured either 4 or 16 hr after the injection of 2-^{14}C-methyldopa or 2-^{14}C-6-hydroxydopamine, was considerably greater when *d*-α-methyltyrosine was also given (Tables 3 and 4). This effect was evident not only in brain and heart but also in liver and kidney. *l*-α-Methyltyrosine, *d*-tyrosine, and *l*-tyrosine, each injected at a dose of 75 mg/kg, had no effect on tissue radioactivity after ^{14}C-methyldopa administration ($P > 0{\cdot}25$).

* Under these conditions most of the tyrosine present is destroyed without serious destruction of AMT.

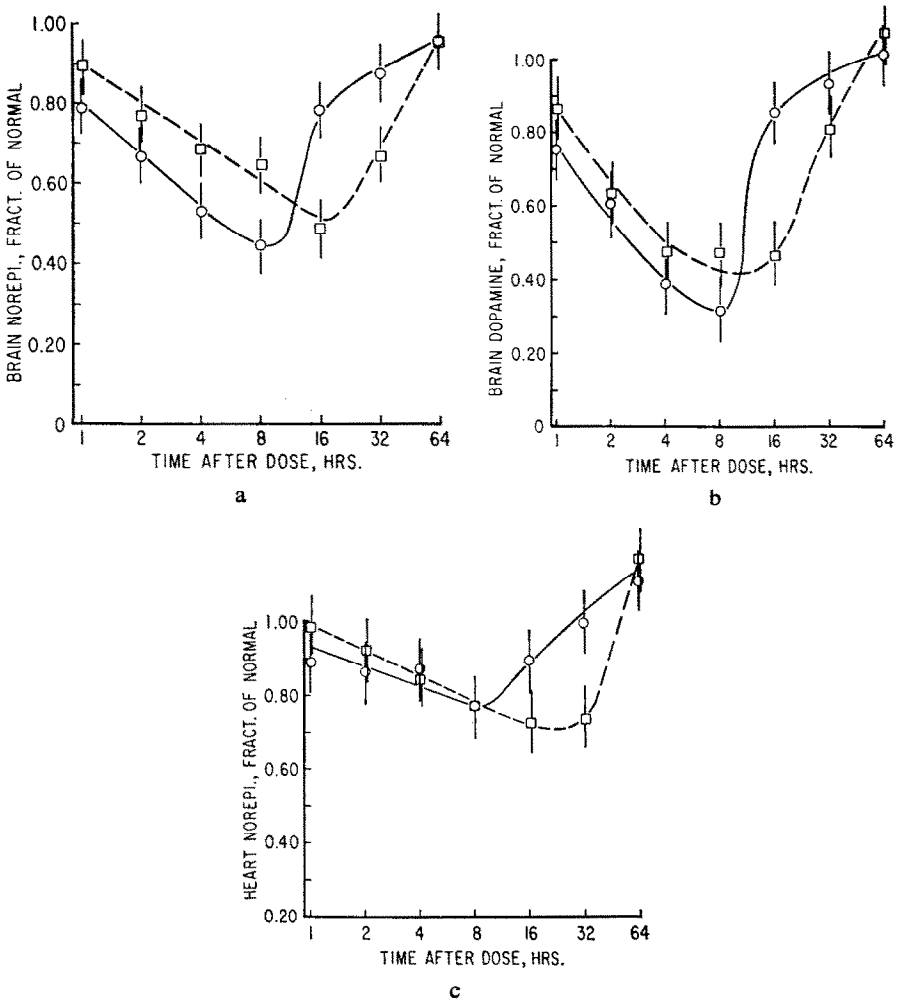

FIG. 1. Tissue catecholamines after administration of α-methyltyrosine. ○, 75 mg/kg, *l*-isomer; □, 50 mg/kg, racemate. Vertical bars represent standard deviations of means derived from the analysis of variance. Three groups of 5 mice per point.

TABLE 1. CATECHOLAMINES IN TISSUES 16 HOURS AFTER *l*-α-METHYLTYROSINE ADMINISTRATION; DEPENDENCE OF POTENTIATION BY *d*-α-METHYLTYROSINE UPON DOSE

Dose of *l*-isomer (mg/kg)	Dose of *d*-isomer (mg/kg)	Tissue catecholamine conc.* (fract. of normal) Brain nor.	Brain dopam.	Heart nor.
18·8	0	1·042	1·048	0·959
18·8	9·4	0·967	0·959	0·969
18·8	18·8	0·729	0·758	0·784
18·8	37·5	0·387	0·496	0·594
18·8	75·0	0·369	0·465	0·657

* Three groups of 5 mice per treatment. Standard deviation from analysis of variance, s = 0·081

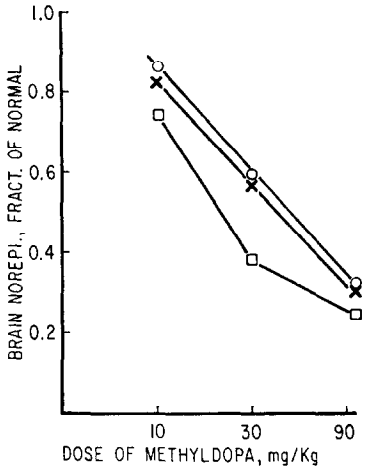

FIG. 2. Potentiation by *d*-AMT of norepinephrine depletion in brain by methyldopa. ○, methyldopa; ×, *l*-AMT, 30 mg/kg; □, *d*-AMT, 30 mg/kg. Brain amine measured 16 hr after i.p. dose. Methyldopa curve, 21 groups of 5 mice; combination doses, 9 groups of 5 mice each curve.

Analysis of variance: methyldopa alone vs. methyldopa and *d*-AMT

Source of var.	df	ss	ms	F	*P*
Total	29	1·550172			
Dose of methyldopa	2	1·379053	0·689527	415·00	<0·001
d-AMT	1	0·059335	0·059335	35·71	<0·001
Dose × *d*-AMT	2	0·071909	0·035955	21·64	<0·001
Error	24	0·039875	0·0016615		
		s = 0·041			

Analysis of variance: methyldopa alone vs. methyldopa and *l*-AMT

Source of var.	df	ss	ms	F	*P*
Total	29	1·492455			
Dose of α-methyldopa	2	1·446989	0·723495	420·64	<0·001
l-AMT	1	0·001075	0·001075	<1	>0·25
Dose × *l*-AMT	2	0·003121	0·001562	<1	>0·25
Error	24	0·041270	0·001720		
		s = 0·041			

The brains and hearts of mice which were treated with *l*-AMT-^{3}H contained more radioactivity when an equal amount of unlabeled *d*-AMT was also injected (Table 5). Although the effect of *d*-AMT was evident at all times observed, it was most striking 8 and 16 hr after administration of the compounds.

^{14}C-Concentrations in brains and hearts of mice 2 hr after labeled methyldopa injections were higher if the animals had also received *d*-α-methyltyrosine 16 hr earlier (Table 6). However, the administration of methyldopa did not affect the concentrations of chemically determined *d*-α-methyltyrosine in the tissues.

DISCUSSION

α-Methyltyrosine administration to mice resulted in a decrease in the concentration of brain dopamine at least equal to the decrease of brain norepinephrine. This is a distinguishing property of the compound as a ring hydroxylation inhibitor since, after the administration of catecholamine depletors to animals, brain norepinephrine concentrations generally decrease more noticeably than do dopamine concentrations.

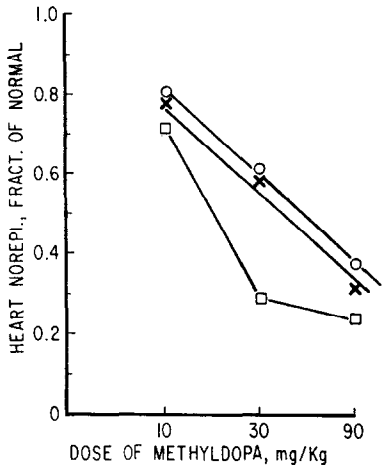

FIG. 3. Potentiation by *d*-AMT of norepinephrine depletion in heart by methyldopa. See Fig. 2 Same experimental conditions except 24 groups of 5 mice were used for methyldopa curve.

Analysis of variance: methyldopa alone vs. methyldopa and *d*-AMT

Source of var.	df	ss	ms	F	*P*
Total	35	1·617576			
Dose of methyldopa	2	0·990309	0·495155	77·72	<0·001
d-AMT	1	0·283002	0·283002	44·42	<0·001
Dose × *d*-AMT	2	0·153137	0·076569	12·02	<0·001
Error	30	0·191128	0·006371		
		s = 0·079			

Analysis of variance: methyldopa alone vs. methyldopa and *l*-AMT

Source of var.	df	ss	ms	F	*P*
Total	35	1·187097			
Dose of methyldopa	2	1·000125	0·500625	77·16	<0·001
l-AMT	1	0·001209	0·001209	<1	>0·25
Dose × *l*-AMT	2	0·010599	0·005300	<1	>0·25
Error	30	0·175164	0·006488		
		s = 0·081			

The interaction of *d*-AMT with *l*-AMT as well as with some catecholamine-depleting agents (6-hydroxydopamine, methyldopa and α-methyl-*m*-tyrosine) is associated with the ability of *d*-AMT to promote higher and more persistent concentrations of the active compounds in the tissues, including not only brain and heart but also liver, kidneys, and presumably other tissues. *d*-AMT may alter transport, metabolism, or excretion of the compounds whose activity it potentiates. Although an unequivocal choice of mechanism cannot be made at present, the following considerations suggest that altered permeability may be an important factor.

First, *d*-AMT has no effect upon tissue catecholamine concentrations. Therefore, it seems unlikely that it profoundly alters the metabolism of the catecholamines and closely related compounds such as 6-hydroxydopamine and methyldopa. Also, *d*-AMT does not potentiate the catecholamine-depleting potency of another closely related compound, metaraminol. It has been suggested that, in all probability, the long-lasting depletion of tissue norepinephrine following administration of methyldopa or α-methyl-*m*-tyrosine is produced by their respective metabolites; viz. α-methylnorepinephrine and metaraminol.[8–10] Thus, since *d*-AMT potentiates the

TABLE 2. EFFECT OF *d*-α-METHYLTYROSINE UPON THE POTENCY OF CATECHOLAMINE DEPLETORS IN DECREASING TISSUE* NOREPINEPHRINE CONCENTRATION, MEASURED 16 HOURS AFTER DOSING

A. *l*-α-Methyl-*m*-tyrosine

Brain†	Without *d*-AMT	With *d*-AMT (30 mg/kg)
ED_{50} of *l*-AMT (mg/kg)	18·24‡	5·14§
95% Confidence limits	11·94, 27·86	3·65, 7·24
Slope¶	−0·245	−0·235

* Since the heart is more sensitive than brain to the depleting effect of α-methyl-*m*-tyrosine,[3] at the higher dose used (30 mg/kg) norepinephrine was depleted over 90% whether or not *d*-AMT was given. At a dose of *l*-AMT, 3 mg/kg, heart norepinephrine was 0·341 of normal (in the absence of *d*-AMT) or 0·162 of normal (in the presence of *d*-AMT, 30 mg/kg); s = 0·032; *P* for effect of *d*-AMT < 0·001; 9 groups of 5 mice.

† Two-dose assay (3 and 30 mg/kg).

‡ Nine groups of 5 mice.

§ Six groups of 5 mice.

¶ Slope of regression line, fraction of normal norepinephrine in tissue on log dose in mg/kg.

B. 6-Hydroxydopamine

	Without *d*-AMT	With *d*-AMT*
ED_{50} of 6-HD (mg/kg)	2·97†	1·80‡
95% Confidence limits	2·72, 3·24	1·66, 1·95
Slope§	−0·934	−1·163

* *P* for effect of *d*-AMT < 0·001.

† Four-point assay; 27 groups of 5 mice.

‡ Three-point assay; 9 groups of 5 mice.

§ Slope of regression line, norepinephrine as fraction of normal on log dose, mg/kg.

TABLE 3. RADIOACTIVITY IN TISSUES AFTER ADMINISTRATION OF METHYLDOPA-2-^{14}C TO MICE: EFFECT OF *d*-α-METHYLTYROSINE

	Radioactivity in tissue (as methyldopa, μg/g)*					
Time after dose (hr)	4		16		s†	*P*‡
d-α-Methyltyrosine (30 mg/kg i.p.)	0	+	0	+		
Tissue						
Brain	1·39	8·20	0·64	2·92	0·94	<0·001
Heart	1·16	11·60	0·61	5·44	1·16	<0·001
Liver	0·49	7·43	0·17	6·32	0·86	<0·001
Kidneys	5·14	131·33	0·67	27·98	0·69	<0·001

* Three groups of 5 mice per treatment; dose of methyldopa, 30 mg/kg i.p.

† Standard deviation from analysis of variance with 8 degrees of freedom within groups.

‡ *P* for effect of *d*-α-methyltyrosine. *P* for time difference, <0·001 (brain, heart, kidneys), >0·10 (liver). Time × drug interaction is significant; *P* <0·001 to <0·005 except for liver (*P* >0·25). If logs of data are used for analysis of variance, the interactions become less significant, or not significant, indicating the geometric relationships involved.

TABLE 4. RADIOACTIVITY IN TISSUES AFTER ADMINISTRATION OF 6-HYDROXYDOPAMINE-2-^{14}C TO MICE: EFFECT OF *d*-α-METHYLTYROSINE

Time after dose (hr)	Radioactivity in tissue* (as 6-OH-dopam., μg/g) 4		16		s†	P‡
d-α-Methyltyrosine (30 mg/kg i.p.)	0	+	0	+		
Tissue						
Brain	0·026	0·080	0·018	0·026	0·021	<0·005
Heart	0·89	1·61	0·34	0·61	0·21	<0·001
Liver	0·73	1·13	0·12	0·26	0·25	<0·05
Kidneys	1·01	7·81	0·43	0·50	4·10	<0·025

* Five mice per treatment; dose of 6-hydroxydopamine, 3 mg/kg i.p.

† Standard deviation from analysis of variance, with 16 degrees of freedom within groups.

‡ *P* for effect of α-methyltyrosine. *P* for time difference, <0·001 (heart and livers), <0·025 (brain and kidneys). *P* for drug × time interaction, >0·05 (brain and hearts), >0·25 (livers), <0·05 (kidneys).

TABLE 5. RADIOACTIVITY IN TISSUES AFTER ADMINISTRATION OF *l*-AMT-^{3}H TO MICE: EFFECT OF *d*-AMT

Tissue	Time after dose (hr)	Radioactivity in tissue calcd. as AMT (μg/g*) ^{3}H-*l*-AMT, 37·5 mg/kg	^{3}H-*l*-AMT, 37·5 mg/kg *d*-AMT, 37·5 mg/kg
Brain	2	14·5	19·7
	4	10·9	22·0
	8	4·2	21·0
	16	0·9	13·3
Heart	2	16·3	26·4
	4	11·7	25·2
	8	4·0	24·3
	16	0·8	22·8

* Three groups of 5 mice per treatment. Standard deviation, from analysis of variance, s = 1·3.

activity of α-methyl-*m*-tyrosine, but not of metaraminol, assuming an effect of *d*-AMT upon metabolism would involve a contradiction.

Second, although data concerning the effect of *d*-AMT on the mouse kidney are not available, in the dog the compound does not influence the clearance of methyldopa by the kidneys.[11] The accumulation of methyldopa in the kidneys, particularly in the presence of *d*-AMT, is striking. Four hours after administration of methyldopa and *d*-AMT, the concentration of methyldopa and its metabolites in the kidneys (131 μg/g) was four times the value predicted (30 μg/g) from the dose of methyldopa given, if equal distribution in the tissues and no excretion are assumed. However, accumulation of a substance in the whole kidney cannot be equated with depressed clearance of the substance. Thus, there is no evidence at present that *d*-AMT affects kidney function.

Third, the mechanism of exchange diffusion[12,13] seems not to be involved. If it were, administration of ^{14}C-methyldopa to animals which were predosed with *d*-AMT should have resulted in displacement of tissue *d*-AMT by radioactive compounds;

TABLE 6. EFFECT OF *d*-AMT PREDOSE UPON RADIOACTIVITY IN TISSUES AFTER ^{14}C-METHYLDOPA ADMINISTRATION

Dose*		Brain		Heart	
d-AMT†	Methyldopa‡	*d*-AMT	^{14}C as methyldopa	*d*-AMT	^{14}C as methyldopa
(mg/kg)		(μg/g)		(μg/g)	
0	0	1·91		0·76	
30	200		44·7		66·9
50	0	9·94		30·81	
0	200	1·55	30·7	2·13	46·0
50	200	8·16	54·0	32·67	102·4
s§		1·34	3·8	2·98	10·0
P¶		>0·05		>0·25	
P‖			>0·001		>0·001

* Three to six groups of 5 mice per treatment.
† Injected 16 hr before methyldopa.
‡ Injected 2 hr before tissues were assayed.
§ Standard deviation from analysis of variance.
¶ *P* for effect of methyldopa on *d*-AMT in tissue.
‖ *P* for effect of *d*-AMT on methyldopa in tissue.

and although more radioactivity was found in the tissues of the predosed mice, the administration of methyldopa did not affect the concentration of *d*-AMT in the tissues.

Thus, it appears most reasonable to conclude that *d*-AMT affects the permeability of tissue *l*-AMT, methyldopa, α-methyl-*m*-tyrosine, 6-hydroxydopamine, and/or their metabolites.

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