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EFFECT OF α-METHYL-5-HYDROXYTRYPTOPHAN ETHYL ESTER UPON TISSUE NOREPINEPHRINE LEVELS IN RATS AND MICE

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Abstract—α-Methyl-5-hydroxytryptophan (α-Me-5-HTP) has been shown to inhibit tyrosine hydroxylase *in vivo*, resulting in a decrease of tissue norepinephrine (NE) levels. Previously unreported studies had demonstrated that the compound was poorly absorbed after oral administration. The ethyl ester of α-Me-5-HTP is an orally active form, as indicated by blood levels of the compound and confirmed by its NE-depleting effect in mouse brain and rat brain, myocardium and adrenals. With single or multiple oral dosing schedules, which deplete rat myocardial NE to as low as 10 per cent of control, brain NE was lowered by 50 per cent. After these chronic dosing procedures, rat brain stem tyrosine hydroxylase was reduced only 20 per cent. Dose (50–400 mg/kg) and time (88–144 hr) studies with single oral doses showed that myocardial NE was markedly depleted at 24 hr by 50 mg/kg and that these NE levels were still below control level 144 hr after 400 mg/kg. The time study in rat brain and myocardium is compared with that produced by α-methyltyrosine methyl ester, 400 mg/kg, p.o. Depletion in myocardium and the adrenal is marked by an initial rapid decline which exceeds that expected to occur after a total blockade of biosynthesis through inhibition of tyrosine hydroxylation. Other possible actions of the compound are discussed.

α-METHYL-5-HYDROXYTRYPTOPHAN (α-Me-5-HTP) inhibits the enzymic hydroxylation of tyrosine *in vitro* and *in vivo*.[1, 2] As with other inhibitors of this enzyme, tissue levels *in vivo* of norepinephrine (NE) are reduced after parenteral administration of α-Me-5-HTP.[1, 2] However, after oral dosing, this compound was only poorly absorbed in man and in monkey, and blood levels of the drug were not detected.* Since the decrease of NE resulting from the inhibition of tyrosine hydroxylase would have potential pharmacological utility,[3] a form of the inhibitor which would be orally active was sought.

This report describes the effects of the ethyl ester of α-Me-5-HTP upon rat and mouse tissue NE levels after its oral administration in several time and dose studies.

METHODS

The ethyl ester of α-Me-5-HTP was prepared by a catalytic reduction of ethyl-α-nitro-β-[3-(5-benzyloxy-indolyl)] propionate.[4]† α-DL-Methyltyrosine methyl ester (α-MT) used in this study was prepared by B. A. Johnson, The Upjohn Company, or was obtained from Regis Chemical Company.

Blood drug levels were done in Carworth Farms mice (18–22 g) which had received

* A. Sjoerdsma; F. S. Eberts and P. H. Seay; personal communication.
† T. L. Lemke, unpublished observations.

α-Me-5-HTP, i.p., or α-Me-5-HTP ethyl ester, p.o. or i.p., 400 mg/kg. Mice were decapitated at various times and blood was collected in heparinized beakers from which 2 ml blood was transferred to a centrifuge tube and centrifuged for 10 min. An aliquot of the plasma (0·75 ml) was removed and diluted with 2 ml water. Proteins were precipitated by the addition of 0·3 ml of 40% trichloroacetic acid and centrifugation. The supernatant was transferred to a 40-ml screw-cap test tube and washed twice with 5 ml diethyl ether. The ether was discarded and the aqueous aliquot diluted to 1 ml with concentrated HCl. Fluorescence of the solution was read in an Aminco–Bowman spectrophotometer at an activation wavelength of 300 mμ and fluorescence wavelength of 535 mμ (uncorrected). A standard curve was prepared for α-Me-5-HTP. Recovery of the standard from the blood was 88–90 per cent.

α-Me-5-HTP, α-Me-5-HTP ethyl ester and α-methyltyrosine methyl ester (α-MT) were suspended in ethyl alcohol–0·25% aqueous methyl cellulose (1:14) and, unless otherwise specified, administered orally to Upjohn Sprague–Dawley male rats, 110–150 g, or to Carworth Farms male mice, 18–22 g. Rats were sacrificed by decapitation and brains were removed and placed upon dry ice. Individual rat brains were homogenized and NE extracted.[5] An aliquot of the repartitioned aqueous extract was assayed for NE.[6] NE content in paired mouse brains was determined in the same manner. Rat hearts were exsanguinated in cold saline and the atrium was removed as completely as possible. The remaining myocardium was blotted, minced and placed upon dry ice. Myocardial tissue was homogenized in 4 ml of 0·4 M perchloric acid and centrifuged. Catecholamines were absorbed on to alumina,[7] and then eluted with 2 m of 0·5 N acetic acid. NE was determined in an aliquot of the eluate.[6] In a single study, rat adrenal NE was similarly determined.

In other studies employing rat brain stem tissue, the cortex and cerebellum were carefully removed from the whole brain immediately after sacrifice. The remaining tissue was labeled "brain stem". Tyrosine hydroxylase activity in the brain stem was determined by the method of McGeer and McGeer.[8] In a second study with brain stem tissue, NE was determined as described above for whole brain. Dopamine (DA) was also determined in a second aliquot of the final aqueous extract.[9]

RESULTS

As indicated earlier, blood levels of α-Me-5-HTP were not detected after oral administration of the compound in man or in monkey. Absorption of the ethyl ester of α-Me-5-HTP was indicated by the blood levels of apparent α-Me-5-HTP detected after both oral and i.p. administration of the ester to mice. A time study of the blood levels of βα-Me-5-HTP in mice demonstrated rapidly falling blood levels of α-Me-5-HTP 1–3 hr after administration of the ester (Fig. 1). However, significant levels (>10 μg/ml) of the drug still remained after 3 hr.

Effectiveness of the absorption of the inhibitor in mice also was demonstrated by the depletion of mouse brain NE. Both α-Me-5-HTP and the ethyl ester, i.p., lowered mouse brain NE with the doses used (Table 1). The ester, 400 mg/kg, p.o., was equally as effective by this route as it was parenterally. Mouse heart NE was not determined in this study. α-Me-5-HTP at 350 mg/kg, p.o., also decreased rat tissue NE levels (Table 1). Orally, the acid was not as effective as an equimolar dose of the ester in reducing myocardial NE. Both forms were equally effective by either route in depleting rat brain NE. All subsequent studies in this report were done in the rat.

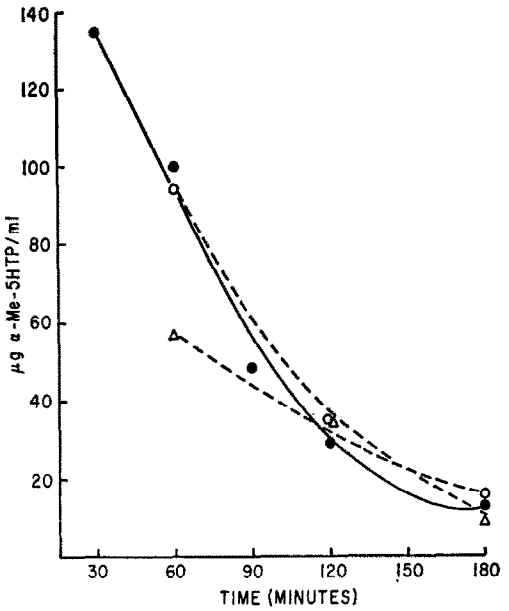

FIG. 1. Mouse blood levels of α-Me-5-HTP after administration of α-Me-5-HTP or α-Me-5-HTP ethyl ester. α-Me-5-HTP, 400 mg/kg, i.p. (— ● —); α-Me-5-HTP ethyl ester, 400 mg/kg, i.p. (– – ○ – –); 400 mg/kg, p.o. (– – △ – –).

TABLE 1. DEPLETION OF MOUSE BRAIN NE AND RAT TISSUE NE AFTER α-ME-5-HTP AND α-ME-5-HTP ETHYL ESTER, I.P. AND P.O.

Experiment	Drug	(mg/kg)	Route administration	(hr)	Norepinephrine (μg/g ± S.E.M.) Brain	Myocardium
1. Mouse	Control	—	—	—	0·46 ± 0·01*	—
	α-Me-5-HTP	500	i.p.	4	0·34 ± 0·05	—
	α-Me-5-HTP ethyl ester	400	i.p.	4	0·27 ± 0·01	—
		400	p.o.	4	0·30 ± 0·01	—
		400	p.o.	8	0·32 ± 0·02	—
2. Rat	Control	—	—	7	0·33 ± 0·01	1·05 ± 0·07
	α-Me-5-HTP	350	i.p.	7	0·19 ± 0·01	0·16 ± 0·02
	α-Me-5-HTP ethyl ester	400	i.p.	7	0·21 ± 0·01	0·18 ± 0·03
3. Rat	Control			7	0·36 ± 0·01	0·83 ± 0·03
	α-Me-5-HTP	350	p.o.	7	0·28 ± 0·01	0·55 ± 0·06
	α-Me-5-HTP ethyl ester	400	p.o.	7	0·27 ± 0·01	0·29 ± 0·08

* Each value is the average of at least three determinations.

The effect of varying single doses of α-Me-5-HTP ethyl ester upon NE levels in rat brain and myocardium is shown in Table 2. Each of the oral doses significantly reduced myocardial levels of NE at both 8 and 24 hr. Brain NE concentrations were diminished only slightly at the highest doses. Results of time study with α-Me-5-HTP ethyl ester, 400 mg/kg, p.o., upon rat brain and myocardial NE levels are listed in Table 3. Maximum depletion of myocardial NE to 18% of control level was detected

TABLE 2. EFFECT OF α-ME-5-HTP ETHYL ESTER AT VARYING DOSES ON RAT BRAIN AND MYOCARDIAL NE LEVELS

	Dose (mg/kg)	Time (hr)	Norepinephrine (μg/g of tissue ± S.E.M.) Brain	Myocardium
Control	—	—	0·33 ± 0·01*	1·08 ± 0·03*
α-Me-5-HTP ethyl ester p.o.	100	8	0·32 ± 0·01	0·56 ± 0·08
	200	8	0·26 ± 0·01	0·34 ± 0·02
	400	8	0·26 ± 0·01	0·31 ± 0·03
	50	24	0·30 ± 0·01	0·58 ± 0·11
	100	24	0·30 ± 0·01	0·44 ± 0·08
	200	24	0·26 ± 0·01	0·35 ± 0·07
	400	24	0·25 ± 0·01	0·19 ± 0·03

* Each value is the average of at least three determinations.

TABLE 3. TIME STUDY OF THE EFFECT OF α-ME-5-HTP ETHYL ESTER ON RAT BRAIN AND MYOCARDIAL NE LEVELS

	(hr)	Norepinephrine (μg/g of tissue ± S.E.M.) Brain	Myocardium
Control	—	0·33 ± 0·01*	1·08 ± 0·03*
α-Me-5-HTP ethyl ester, 400 mg/kg, p.o.	4	0·29 ± 0·02	
	8	0·26 ± 0·01	0·31 ± 0·03
	16	0·26 ± 0·01	0·30 ± 0·04
	24	0·25 ± 0·01	0·19 ± 0·03
	48	0·29 ± 0·01	0·48 ± 0·04
	96	0·29 ± 0·01	0·70 ± 0·12
	144	0·29 ± 0·01	0·82 ± 0·01

* Each value is the average of at least three determinations.

TABLE 4. EFFECT OF MULTIPLE DOSES OF α-ME-5-HTP ETHYL ESTER ON RAT BRAIN, MYOCARDIAL AND ADRENAL NE LEVELS

	Dose (mg/kg)	Dosing (hr)	Sacrifice (hr)	Brain	Myocardium	Adrenal
Control	—	—	—	0·33 ± 0·01*	1·08 ± 0·03	800 ± 90
α-Me-5-HTP Ethyl ester	100	0, 24, 48	56	0·18 ± 0·01	0·11 ± 0·02	—
	400	0, 4	8	0·24 ± 0·01	0·35 ± 0·07	420 ± 20
	400	0, 4	24	0·20 ± 0·01	0·15 ± 0·02	—
	400	0, 4	48	0·25 ± 0·01	0·47 ± 0·07	—
	400	0, 4	96	0·26 ± 0·01	0·81 ± 0·06	—
	400	0, 4, 8	24	0·16 ± 0·01	0·08 ± 0·02	470 ± 30
	400	0, 4, 8	48	0·25 ± 0·01	0·36 ± 0·05	—

* Each value is the average of at least three determinations.

at 24 hr. NE concentrations in the myocardium had returned 75% to of control level 6 days after administration of the drug. In these same rats, brain NE levels were only slightly altered and fell only to 75 per cent of the control level in the first 24 hr.

Table 4 summarizes the results of multiple dosing of α-Me-5-HTP ethyl ester upon rat myocardial, brain and adrenal NE levels. Tissue levels were significantly reduced

by the various regimen employed. Near total depletion of NE occurred in rat myocardium 24 hr after dosing with 400 mg/kg at 0, 4 and 8 hr. Chronic dosing with 100 mg/kg also reduced endogenous myocardial stores to 10 per cent of control level. Both of these dosing schedules also lowered brain NE to approximately 50 per cent of control. NE in the rat brain stem was also reduced by approximately 50 per cent (0·54 ± 0·02 μg/g to 0·29 ± 0·01 μg/g) by the former chronic dosing schedules. In time studies of greater than 24 hr in duration, multiple dosing at 400 mg/kg in the first day was no more effective than a single dose in reducing tissue NE. Toxicity or altered overt behavior was not encountered in any of the studies in this investigation with α-Me-5-HTP ethyl ester.

In an attempt to determine the length of time required to replete rat myocardial NE stores after chronic dosing, α-Me-5-HTP ethyl ester (50 mg/kg, once a day) was given for 7 days. One day after the end of dosing, myocardial NE was reduced to 45 per cent of control (0·93 ± 0·11 μg/g). Brain and adrenal NE stores were not altered by this regimen. Myocardial stores had returned to 72 per cent of control 7 days after the termination of drug treatment and were at control level after 14 days

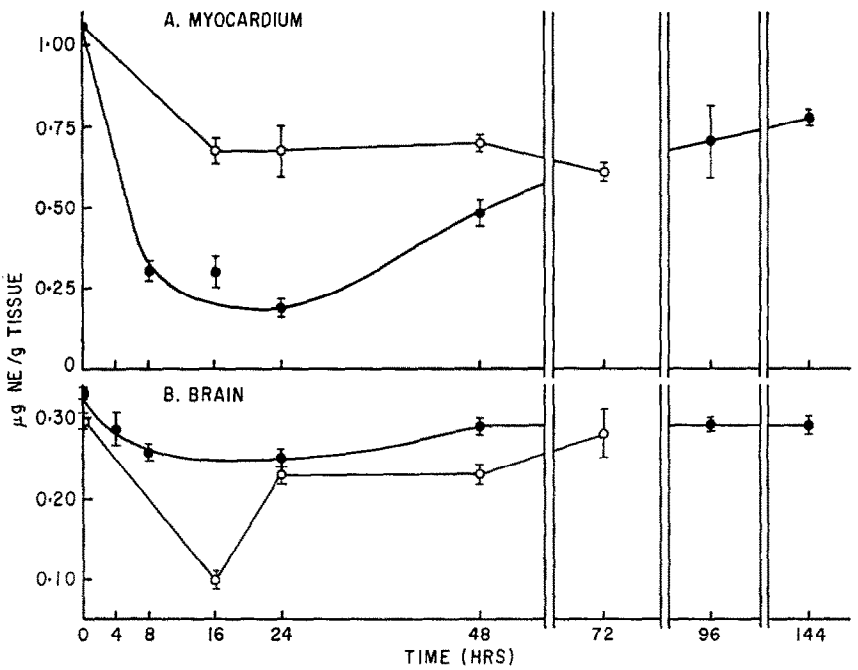

FIG. 2. Comparison of time studies of NE depletion in rat brain and myocardium after α-Me-5-HTP ethyl ester (— ● —) and α-MT (— ○ —); both compounds administered at 400 mg/kg ,p.o.

Depletion of brain and myocardial NE levels after single oral doses of either α-Me-5-HTP ethyl ester or α-MT is compared in Fig. 2. α-MT (400 mg/kg) produced maximum depletion in both brain and myocardium at 16 hr The single dose of α-MT, which had some toxicity associated with it, was much more effective in lowering brain NE levels than the α-Me-5-HTP ethyl ester. On the other hand, α-MT was not as effective as α-Me-5-HTP ethyl ester in depleting NE from the myocardium.

Repletion of brain NE to control levels by 72 hr after α-MT was noted, whereas myocardial NE remained substantially below control concentrations.

The extent that brain tyrosine hydroxylase is inhibited by chronic α-Me-5-HTP pretreatment can best be assessed by measuring enzyme activity in the brain stem.[8] After the dosage regimen which produced maximal depletion of rat brain NE (400 mg/kg at 0, 4 and 8 hr; sacrifice at 24 hr; Table 4), rat brain stem tyrosine hydroxylase activity was diminished only 19 per cent (19 $\pm$ 8%, n = 3). Accordingly, under the same dosing schedule rat brain stem NE levels were decreased 45 per cent, the same extent of NE depletion measured in whole brain. Brain stem DA was not altered from control levels.

DISCUSSION

An increased emphasis upon the role of catecholamines in the function of cardiovascular and central nervous systems has stimulated interest in compounds which alter tissue levels of these endogenous compounds. The mechanism for these changes includes: (1) inhibition of the biosynthesis of the catecholamines, and (2) a drug-mediated release of the endogenous amines from their binding site. Primary attention in the blockade of biosynthesis has been focused upon the rate-limiting step in the scheme, i.e. the inhibition of tyrosine hydroxylase.[10] Among the compounds which inhibit this enzyme competitively are various derivatives of tyrosine, including the α-methyl[11] and 3-iodo[12] homologs. A number of other compounds which are non-competitive inhibitors of this enzyme *in vitro* include dopacetamide (H 22/54),[13] the arterenones[14, 15] and several pyrroloisoxazoles.[16] While McGeer *et al.*[17] have demonstrated the competitive inhibition of tyrosine hydroxylase *in vitro* by 5-halotryptophans, Zhelyazkov *et al.*[1] have reported the noncompetitive inhibition of this enzyme with α-Me-5-HTP. Although the exact mechanism of the inhibition by α-Me-5-HTP has not been clearly determined, the inhibition is not reversible by increasing concentrations of the amino acid substrate. Thus inhibition *in vivo* of tyrosine hydroxylase by α-Me-5-HTP would not be affected by increased dietary intake of tyrosine.

The detected blood levels of α-Me-5-HTP after oral administration of the ethyl ester of this compound (Fig. 1) and the depleting activity produced by the ester and by α-Me-5-HTP, i.p., in mice and rats (Table 1) indicate that the ethyl ester of α-Me-5-HTP is an orally active form of the acidic indole.

Data from these studies demonstrated a greater susceptibility of the rat heart to the NE-depleting activity of α-Me-5-HTP than either the whole brain or the brain stem. Only under chronic dosing procedures at 400 mg/kg, which depleted rat myocardial NE to approximately 10 per cent of control, did depletion of rat brain or brain stem approach 50 per cent (Table 4). In contrast, a single 50 mg/kg dose of the ester depleted myocardial NE to 50 per cent after 24 hr. This extent of NE depletion was also achieved with a single dose of 100 mg/kg after 8 hr (Table 2). Maximal depletion of myocardial NE levels (18 per cent of control) at 24 hr with a single 400 mg/kg dose was accompanied by only a token decline of rat brain NE levels (% 25 per cent of control; Table 2).

Zhelyazkov *et al.*[1] have previously demonstrated that α-Me-5-HTP (200 mg/kg, i.p.) produced an inhibition of tyrosine hydroxylase activity *in vivo* in guinea pig heart press juice which remained substantially reduced for 48 hr. The duration of the inhibition and the depletion of rat myocardial NE in this study (Fig. 2) resemble

the results reported for the inhibition of brain tryptophan hydroxylase and the depletion of brain serotonin after *p*-chlorophenylalanine.[18] The irreversible nature of this inhibition is contrasted with the short-term depletion of rat brain NE by α-MT, a competitive inhibitor (Fig. 2). In our efforts to correlate enzyme inhibition and myocardial NE depletion, we attempted without success to assay tyrosine hydroxylase activity in rat myocardial press juice, and in myocardial and whole heart slices utilizing tyrosine-^{14}C and tyrosine 3,5-^{3}H as substrates. Enzyme activity in guinea pig heart press juice[14] was obtained. Inhibition *in vivo* of brain tyrosine hydroxylase by chronic dosing with α-Me-5-HTP ethyl ester reached only 20 per cent. The inability of α-Me-5-HTP to effectively inhibit the brain enzyme was substantiated by the resistance of rat brain NE to depletion and by the maintained NE and DA levels in rat brain and brain stem.

Reasons for the tissue selectivity in the depleting action of α-Me-5-HTP are not entirely clear. Permeability (blood–brain barrier) and differences in the particulate nature of the brain and heart enzymes as previously proposed[1] may explain these differences in susceptibility. NE depletion of rat brain after only large doses of 5-HTP[19, 20] may indicate a specificity in the uptake of the indole into the NE containing nerve terminals in the brain. This may account for the protection of only brain serotonin stores by 5-HTP after reserpine.* The ability of various indoles to release NE-H^{3} from mouse heart[21] may also indicate a lesser specificity in the uptake of the indoles into the sympathetic nerve endings of peripheral tissue. The decline of rat myocardial NE, with a turnover rate or half-life ($T_{\frac{1}{2}}$) of 12 hr,[22] was more rapid than that attributable only to a disruption of the biosynthesis of the amine. Similarly, the decrease in adrenal NE content to approximately 60 per cent of control in 24 hr (Table 4) is also much more rapid than its $T_{\frac{1}{2}}$ of approximately 7 days.[22] These rapid initial rates of tissue depletion implicate a releasing action of the indole upon peripheral NE stores. Lahti *et al.*[23] have now clearly demonstrated that α-Me-5-HTP is decarboxylated *in vivo* to form α-methyl-serotonin which effectively releases NE from mouse heart.

The present studies then strongly suggest that the NE depleting action of α-Me-5-HTP combines the inhibition of tyrosine hydroxlation and a release of endogenous NE stores from peripheral tissues.

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