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EFFECTS OF α-METHYL-5-HYDROXYTRYPTOPHAN AND α-METHYL-5-HYDROXYTRYPTAMINE ON NOREPINEPHRINE IN MOUSE MYOCARDIUM

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Abstract—α-Methyl-5-hydroxytryptophan was shown to exert a releasing action on mouse heart ^{3}H-norepinephrine and endogenous norepinephrine. This releasing action was blocked by pretreatment with *m*-cresol, α-(1-methylhydrazino)-dihydrogen phosphate (50 mg/kg), a decarboxylase inhibitor, and the releasing action was therefore attributed to the decarboxylation product, α-methyl-5-hydroxytryptamine. α-Methyl-5-hydroxytryptamine (15 mg/kg) was found to deplete heart norepinephrine to 30 per cent of control levels after 8 hr, which was as great a depletion as that obtained with α-methyl-5-hydroxytryptophan (200 mg/kg) after a similar time period. The duration of action of the two compounds was also comparable, norepinephrine returning to approximately 50 per cent of control values in 24 hr. α-Methyl-5-hydroxytryptamine was identified chromatographically in mouse hearts pretreated with α-methyl-5-hydroxytryptophan.

PREVIOUSLY published data[1, 2] have shown that α-methyl-5-hydroxytryptophan (α-Me-5-HTP) is a potent tyrosine hydroxylase inhibitor both *in vitro* and *in vivo*. Such an inhibition of the rate-limiting step[3] of norepinephrine synthesis has been shown to cause a depletion of catecholamine stores.[4] It has also been reported that α-Me-5-HTP is a potent inhibitor of aromatic amino acid decarboxylase.[5] However, an inhibition of this enzyme has been shown to have only a transient effect on catecholamine levels.[6]

This report is concerned with yet another aspect of α-Me-5-HTP action, its decarboxylation and the subsequent effects of α-methyl-5-hydroxytryptamine (α-Me-5-HT) on norepinephrine stores.

EXPERIMENTAL

α-Methyl-5-HTP and its ethyl ester and α-methyl-5-HT were prepared at The Upjohn Co.[5, 7] *m*-Cresol, α-(1-methylhydrazino)-dihydrogen phosphate (NSD-1034) was obtained from Dr. D. J. Drain of Smith and Nephew Pharm., Ltd. α-Methyl-*m*-tyrosine was purchased from the Regis Chemical Co.

Endogenous norepinephrine (NE) was determined by the method of Anton and Sayre[8] or by a modification of the procedure of Bertler,[9] using an Amberlite ion-exchange resin; this latter method was also used for the α-Me-5-HT and 5-hydroxytryptamine (5-HT) determinations.

Weighed samples of myocardium were prepared by homogenizing in 15 ml of 0·4 N $HClO_4$ with a ground-glass homogenizer. The homogenate was centrifuged at

10,000 *g* for 10 min in a Sorval centrifuge. The supernatant was transferred to a 30-ml beaker and 2 drops of Bromo Cresol Purple pH indicator (0·1% in ethanol were added. The solution was stirred with a magnetic stirring bar and the pH was adjusted to 5·2 – 6·8 by the dropwise addition of 5 N K_2CO_3. The solution was then transferred to a glass-stoppered centrifuge tube and samples were centrifuged to sediment the $KClO_4$.

Amberlite CG-50 was prepared for use by the method of Pisano.[10] Columns, 20 × 0·5 cm, were prepared for use by adding a small piece of glass wool to the bottom of the tube, filling the columns with water and adding resin to a height of 2·5 cm. After the water had passed through the column, 2·5 ml of 0·02 M phosphate buffer, pH 6·5, was added and the supernatants from the prepared samples were added to the columns. The $KClO_4$ precipitate was washed with 2 ml H_2O, samples were centrifuged, and the wash was added to the column along with the sample. The column flowed at a rate of approximately 2 ml per min.

Upon passage of the sample through the column, the resin was washed with 10 ml of 0·02 M phosphate buffer. The amines were eluted with 4 ml of 1 N HCl.

For the determination of α-Me-5-HT or 5-HT or both, a 1-ml aliquot of the eluate was removed and transferred to a test tube containing 0·5 ml of concentrated HCl. The samples were mixed and read on an Aminco–Bowman spectrophotofluorometer at an activation of 300 mμ and emission of 540 mμ (uncorrected wavelengths). This was possible because the behavior in the Amberlite procedure and the fluorescent spectra for the two compounds were identical. The values obtained for α-Me-5-HT in the heart after α-Me-5-HTP administration are contaminated by small amounts of 5-HT normally present in mouse heart. To correct for this contaminating 5-HT, the amount found in control animals was subtracted from that found in the treated animals. This is valid if one assumes that α-Me-5-HTP administration has no effect on 5-HT synthesis, storage or degradation. One might anticipate a decreased synthesis of 5-HT due to a competition between α-Me-5-HTP and 5-HTP for the decarboxylating enzyme and an increased release of 5-HT because of a competition for binding sites by α-Me-5-HT. However, both of these would give decreased levels of 5-HT and would not affect the end result. No evidence has been presented to demonstrate an inhibition of monoamine oxidase (MAO) by α-Me-5-HT; nevertheless, the possibility does remain and this could affect the final interpretation of the data.

To the remaining 3 ml of the eluate, 0·9 ml of 1·3 N K_2CO_3 was added dropwise while mixing on a Vortex shaker. This neutralizes the 1 N HCl and gives a final pH of 5·2 to 6·8. A 1·5-ml sample was removed and norepinephrine was determined by the method of von Euler and Floding.[11]

α-Me-5-HT was identified in the eluate from the columns by using thin-layer chromatography (TLC) methods. The column eluate from mice sacrificed 4 hr after treatment with α-Me-5-HT was neutralized with 1·3 N K_2CO_3. The sample was then centrifuged to sediment the $KClO_4$. The supernatant was lyophilized, the residue taken up in 1 ml ethanol: H_2O (1:1) and 100 μl was spotted on an MN cellulose 300 G TLC plate. The plate was prepared and developed by the method of Johnson and Boukma.[12] In this TLC system, α-Me-5-HT moves ahead of 5-HT. The respective R_f values were 0·63 and 0·49. Untreated mouse heart samples were carried through the procedure and served as controls. α-Me-5-HT and 5-HT were located on the TLC plates by spraying the plates with *o*-phthalaldehyde[13] reagent [0·05% in 6 N HCl:

methanol (4:6)]. The sprayed plates were heated at 100° for several minutes and the spots were observed under an ultraviolet lamp.

The effect of α-Me-5-HTP and related drugs on mouse heart ^{3}H-NE was determined by a modification of the method of Daly *et al.*[14] ^{3}H-NE (7·3 c/m-mole, New England Nuclear Corp.) was diluted with Merlis solution prior to intravenous (i.v.) administration. All drugs were dissolved or suspended in 0·25% aqueous methylcellulose. Mice were given 3·0 μc ^{3}H-NE; drug administration and sacrifice times are as described in text. Mice were decapitated at specified times, hearts were excised and rinsed in cold saline, atriums were removed and hearts were frozen on dry ice. The hearts were then weighed and homogenized with a ground-glass homogenizer in 4 ml of cold 0·4 N $HClO_4$. Homogenates were centrifuged at 10,000 *g* in a Sorval centrifuge for 10 min. A 1·5-ml aliquot was removed and placed in a scintillation vial containing 15 ml toluene:Triton X-100 (3:1) scintillation fluid.[15] Samples were counted in a Packard liquid scintillation counter.

RESULTS

Initial studies with α-Me-5-HTP on endogenous mouse myocardial norepinephrine levels (Fig. 1) indicated that the compound may have an effect on norepinephrine other

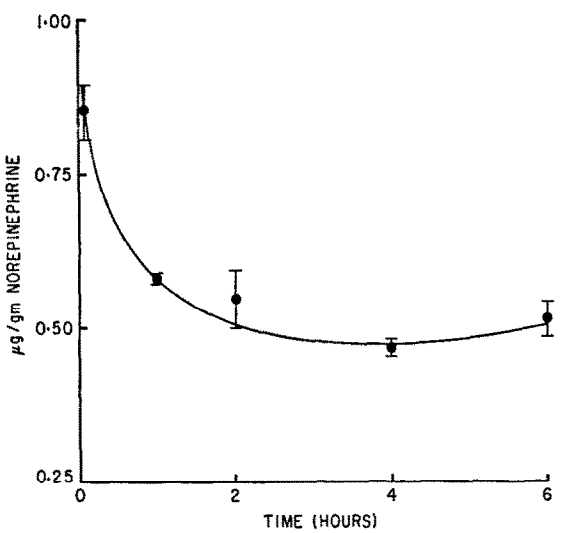

FIG. 1. Effect of 200 mg/kg of α-Me-5-HTP ethyl ester on mouse myocardial norepinephrine levels. The drug was administered orally by stomach tube and the animals were sacrificed at various intervals thereafter. Zero time controls received only vehicle. Each point is mean of two determinations and five hearts were used per determination. Vertical bars represent the range of values.

than that of a tyrosine hydroxylase inhibitor. If the decrease in norepinephrine were due to an inhibition of its synthesis, one would anticipate a slow decline in norepinephrine as it is utilized and metabolized. Contrary to this expected finding, it can be seen that there is a rapid decline in norepinephrine. This rapid decline could be due to a release of NE or α-Me-5-HT, the decarboxylated product of α-Me-5-HTP.

This proposed releasing action was further studied by using ^{3}H-NE to label NE

stores in mouse heart. This method permits a study of the releasing action without interference from the blockade of synthesis. The data presented in Table 1 demonstrate that both α-Me-5-HTP and U-29,262, the ethyl ester of α-Me-5-HTP, cause a release of ^{3}H-NE from the mouse heart. The data also indicate that the ethyl ester of α-Me-5-HTP is as effective orally as α-Me-5-HTP is intraperitoneally (i.p.). α-Me-5-HT is shown to nearly deplete ^{3}H-NE at 50 mg per kg and supports the proposal that it is the actual releaser. A comparable effect was observed on endogenous NE levels (Table 2), as has been reported by others.[16]

TABLE 1. EFFECT OF α-ME-5HTP, α-Me-5-HTP ETHYL ESTER AND α-ME-5-HT ON ^{3}H-NE IN THE MOUSE HEART*

Drug	Dose (mg/kg)	Route	Av. cpm/1·5 ml	% Control
Control	saline	i.p.	2408 (2150–2628)	—
α-Me-5-HTP	200	i.p.	846 (917– 797)	35·1
α-Me-5-HTP	400	i.p.	699 (703– 695)	29·0
α-Me-5-HTP ethyl ester	200	oral	1078 (1056–1110)	44·8
α-Me-5-HTP ethyl ester	400	oral	916 (840–1006)	38·0
α-Me-5-HT	50	i.p.	313 (286– 333)	13·0
α-Me-5-HT	10	i.p.	938 (761–1115)	37·0

* ^{3}H-NE (3·0 μc) was administered i.v. to mice and 1 hr later the drug was given i.p. Animals were sacrificed 3 hr after ^{3}H-NE. Three determinations were obtained for each drug and dose, and two mice were used in each determination. Values enclosed by parentheses are range of values obtained.

TABLE 2. EFFECTS OF α-ME-5-HT ON MOUSE MYOCARDIAL NOREPINEPHRINE*

Dose (mg/kg)	NE (μg/g)	S.D.
—	0·78	± 0·23
15	0·22	± 0·05
50	0·09	± 0·03
100	0·04	± 0·02

* Drug was administered i.p. Mice were sacrificed 4 hr after the drug. Five mice were used per determination and three determinations were made per dose.

Table 3 shows the effects of NSD-1034, an inhibitor of the decarboxylase enzyme,[17] on the releasing action of α-Me-5-HTP. For comparative purposes, α-methyl-*m*-tyrosine (α-Me-m-T) is included, since it has been demonstrated[17] that it is a releaser of NE due to its decarboxylation and formation of α-methyl-*m*-tyramine and metaraminol. It can be observed that NSD-1034 has little effect on ^{3}H-NE levels in mouse heart. However, when it is administered 30 min prior to α-Me-5-HTP or α-Me-m-T, it significantly blocks the releasing action of these drugs. Thus, it appears that decarboxylation of α-Me-5-HTP and the formation of α-Me-5-HT are requisites for it to be a releaser of norepinephrine.

This hypothesis was further tested by following the decline in myocardial

TABLE 3. RELEASE OF ^{3}H-NE FROM MOUSE HEART BY α-ME-5-HTP AND α-ME-M-T AND THE EFFECTS OF DECARBOXYLASE INHIBITION*

Drug	Dose (mg/kg)	NSD-1034 (mg/kg)	cpm/1·5 ml ± S.D.	% Control
—	—	—	2587 ± 111	—
—	—	50	2113 ± 144	81
α-Me-5-HTP	100		1566 ± 97	61
α-Me-5-HTP	100	50	2200 ± 92	85
α-Me-m-T	10		1046 ± 60	40
α-Me-m-T	10	50	2080 ± 89	81

* Mice were given 3·0 μc ^{3}H-NE i.v. A decarboxylase inhibitor, NSD-1034, was given i.p. 30 min after ^{3}H-NE α-Me-5-HTP or α-Me-m-T was administered i.p. 60 min after ^{3}H-NE. Mice were sacrificed 3 hr after ^{3}H-NE. Control animals received vehicle 60 min after ^{3}H-NE. Two mice were used per determination and three determinations were made per drug schedule.

norepinephrine and the increase in apparent α-Me-5-HT in myocardium after α-Me-5-HTP and α-Me-5-HT administration.

Mice were treated with a single dose of 200 mg per kg of α-Me-5-HTP or 15 mg per kg of α-Me-5-HT and sacrificed at the designated times. Three determinations were made for each time interval and five mouse hearts were used per determination. The amount of 5-HT found in control animals was 0·30 μg per g. This value has been subtracted from the amount found in the treated animals.

Figure 2 clearly shows that there is a correlation between the amount of α-Me-5-HT in the heart and the decrease in NE. The NE levels start to return to control values 8 hr after the drug has been administered and have not returned to control levels after 48 hr. α-Me-5-HT reaches a maximum at about 8 hr after its parent amino acid has been administered and is still detectable at 48 hr.

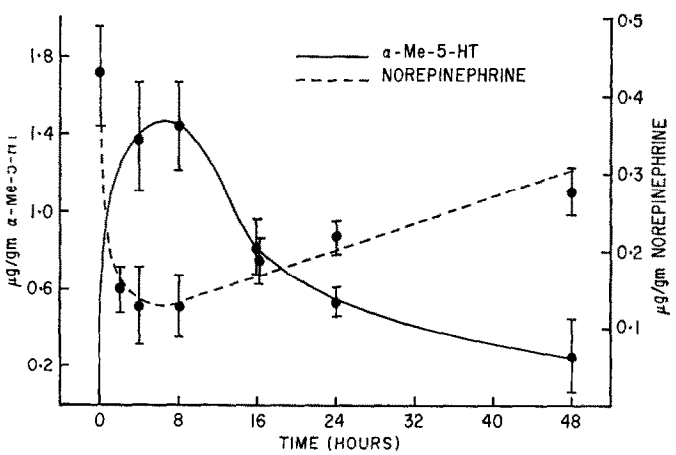

FIG. 2. Norepinephrine and α-Me-5-HT levels in mouse myocardium after the administration of 200 mg/kg of α-Me-5-HTP. Animals were injected i.p. and sacrificed at various times after the drug. Each point is the mean of three determinations using five hearts per determination. Control values for material fluorescing as α-Me-5-HT were 0·30 ± 0·05 μg/g of tissue and have been subtracted from the values obtained from treated animals. Vertical bars represent S.D.

The TLC results confirmed the presence of α-Me-5-HT and 5-HT. Fluorescent spots were observed migrating with R_f values of 0·59 and 0·39. These values are lower than those noted before; however, it was found that authentic samples of 5-HT and α-Me-5-HT, carried through the entire procedure without tissue, gave similar R_f values. The lower R_f values appear to be due to the formation of salts of the amines during their elution from the column.

The data tend to imply that α-Me-5-HT plays a large role in the observed decrease of NE after α-Me-5-HTP administration. However, it is not possible to separate the effect of inhibition of NE synthesis from the releasing action. It does appear that the initial rapid decrease is due to α-Me-5-HT, since an inhibition of synthesis would not cause such a rapid decline. The high levels of α-Me-5-HT are probably the result of decarboxylation of α-Me-5-HTP in tissues other than the heart; thus a large amount of the amine would be formed in a very short time.

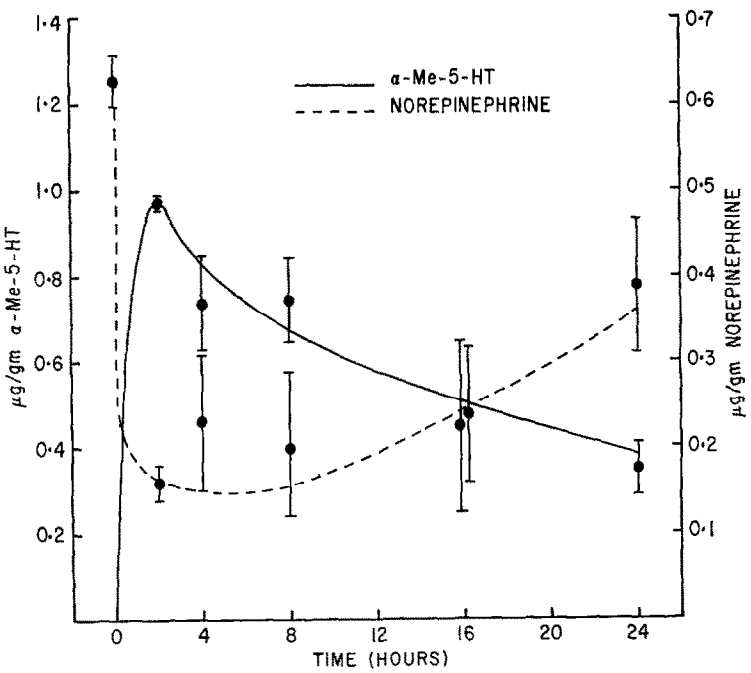

FIG. 3. Norepinephrine and α-Me-5-HT levels in mouse myocardium after the administration of 15 mg/kg of α-Me-5-HT. Animals were injected i.p. and sacrificed at various times after the drug was administered. Each point is the mean of three determinations using five hearts per determination. Control values for material fluorescing as α-Me-5-HT were 0·32 ± 0·04 μg/g of tissue and have been subtracted from the values obtained from treated mice. Vertical bars represent S.D.

The effects of α-Me-5-HT were studied by administering the amine at a dose of 15 mg per kg (i.p.), which decreases NE to values comparable to those observed with 200 mg per kg of α-Me-5-HTP, and following the decline in NE as a function of time. The data presented in Fig. 3 are comparable to those observed in Fig. 2. Differences are seen in a more rapid recovery of NE and in lower α-Me-5-HT levels. The duration of effect on NE is shorter than that observed with α-Me-5-HTP; however, the effect is still of a long duration.

DISCUSSION

The present results indicate that α-Me-5-HTP exerts a releasing action on mouse myocardial norepinephrine stores. The initial rapid decline in NE after a single dose of α-Me-5-HTP (Fig. 1) suggested that an action other than tyrosine hydroxylase inhibition was occurring. This was borne out by the fact that α-Me-5-HTP or its orally active ethyl ester was able to cause a release of ^{3}H-NE from the mouse heart (Table 1). The decarboxylated product of α-Me-5-HTP, α-Me-5-HT, was implicated as the active releasing agent when it was found that a decarboxylase inhibitor, NSD-1034, could block the release of ^{3}H-NE by α-Me-5-HTP. It was further shown that α-Me-5-HT itself could release ^{3}H-NE and endogenous NE, depleting endogenous mouse myocardial stores to 30 per cent of control values within 4 hr. A time study correlating the decline in endogenous NE and the increase in α-Me-5-HT after 200 mg/kg of α-Me-5-HTP demonstrated that, when NE was at its lowest level, α-Me-5-HT was at its highest level and that the decline in α-ME-5-HT corresponded with a return of NE to near control values. A similar time study using a single dose of 15 mg/kg of α-Me-5-HT presented a similar picture. Thus it appears from this study that α-Me-5-HTP reduces mouse myocardial NE stores primarily through the releasing action of its decarboxylated product, α-Me-5-HT.

Initially, it appeared difficult to explain the long duration of action of α-Me-5-HTP[7] on myocardial NE levels by the above hypothesis. However, other investigators[18] have shown that a dose of 20 mg/kg of α-Me-5-HT administered to mice caused an "apparent" increase in 5-HT in blood platelets of approximately 50 per cent. The apparent increase was shown to be due to the injected amine. If one assumes that normal mouse blood platelets contain 5–6 μg 5-HT/ml blood,[18] a 50 per cent increase due to α-Me-5-HT would be 2·5 μg amine/ml blood, which is considerably more α-Me-5-HT than is found in a single mouse heart at its peak level (0·3 μg α-Me-5-HT/mouse heart or 1·4 μg/g of heart tissue). *In vitro*, it was also demonstrated that α-Me-5-HT appeared to be taken up by the same mechanism which transports 5-HT and that this uptake was antagonized by inhibitors in the same manner as was 5-HT.[18] Therefore, it seems that platelets may serve as a reservoir for α-Me-5-HT and prevent its rapid elimination. Coupling the reservoir hypothesis with the fact the α-Me-5-HT is not metabolized[19] by MAO, which is the major catabolic pathway for 5-HT, it is easier to conceive of maintaining high levels of α-Me-5-HT for extended periods. It would seem that the lack of metabolism of MAO and storage of α-Me-5-HT in platelets could provide an available source of α-Me-5-HT, which in turn could diminish and subsequently maintain heart NE stores at a low level.

Any significant difference between α-Me-5-HT and α-Me-5-HTP administration on myocardial NE and α-Me-5-HT levels may be due to the comparatively slow formation of α-Me-5-HT after α-Me-5-HTP administration, which would facilitate the uptake and storage of the amine. Whereas, when α-Me-5-HT is administered, the uptake mechanisms would be rapidly saturated and a large portion of the amine would be quickly eliminated.

The apparent contradiction in the mode of action of α-Me-5-HTP on NE levels, tyrosine hydroxylase inhibition[1, 2] and a release type of mechanism could be species difference. Species with a lower level of decarboxylation of the amino acid would probably exhibit a greater tyrosine hydroxylase inhibition, while in those species which rapidly decarboxylate the amino acid one would probably observe a greater releasing action.

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