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SELECTIVE PROTECTION OF SEROTONIN STORES AGAINST THE ACTION OF RESERPINE BY α-METHYL-5-HYDROXYTRYPTPOHAN PRETREATMENT*

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Abstract—Administration of a single dose of 200 mg/kg of α-methyl-5-hydroxytryptophan to mice results in a rapid decline of brain norepinephrine of approximately 50 per cent and a concomitant increase in "apparent" serotonin of 150–200 per cent. The increase in "apparent" serotonin was attributed to the formation of α-methyl serotonin and was shown to be so by the use of ^{3}H-α-methyl-5-hydroxytryptophan. A time study indicated that after a single dose of the α-methyl amino acid the apparent serotonin levels were elevated above normal for up to 48 hr. Pretreatment of mice with α-methyl-5-hydroxytryptophan prevented the depletion of serotonin stores by reserpine, but did not protect norepinephrine stores. A time study demonstrated that such protection against reserpine is afforded for at least 24 hr after the administration of α-methyl-5-hydroxytryptophan.

α-METHYL-5-HYDROXYTRYPTOPHAN (α-Me-5HTP) has been shown to reduce the content of norepinephrine in the mouse heart,[1] rat heart and brain.[2] The possible mechanism of action of this compound has been shown to be either an inhibition of tyrosine hydroxylase[3] or a releasing action by the decarboxylated product of α-Me-5HTP, α-methyl serotonin (α-Me-5HT).[1]

Recent evidence has indicated that the antihypertensive properties of α-Me-5HTP may be of a central origin and, more specifically, due to α-Me-5HT and not to a decrease in norepinephrine.[4] Although the effects of α-Me-5HTP on norepinephrine are somewhat defined,[1–3] the biochemical properties of α-Me-5HT have not been explored. This study is concerned with the effect of α-Me-5HTP on the brain amines, norepinephrine and serotonin, and especially with the formation of α-Me-5HT and the biochemical effects exerted by it.

METHODS

α-Me-5HTP and α-Me-5HT were prepared at the Upjohn Company.[5] ^{3}H-α-methyl-5-hydroxytryptophan (^{3}H-α-Me-5HTP) with a specific activity of 36·86 mc/m-mole and with >68 per cent of the label in the 2-position and >31 per cent in the 6-position was prepared by Dr. R. S. P. Hsi of the Upjohn Company. Prior to usage, the material was diluted with unlabeled α-Me-5HTP to a specific activity of 2·93 mc/m-mole.

Male Carworth Farm mice weighing 18–20 g were used in all studies. All drugs were administered by the intraperitoneal (i.p.) route.

The amines, serotonin (-5HT), α-Me-5HT and norepinephrine (NE) were isolated

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by homogenizing two brains in 12 ml of 0·4 N $HClO_4$, centrifuging the homogenates, adjusting the pH of the supernatants to 6·5 and passing the neutralized solutions through an Amberlite CG-50 resin as previously described.[1] The amines were eluted from the columns with 4 ml of 1 N HCl. NE was determined by the method of von Euler and Floding[6] and 5-HT and α-Me-5HT by measuring the acid-induced fluorescence.[1] As previously mentioned, α-Me-5HT behaves similarly to 5-HT in the isolation procedure and it also possesses the same fluorescence spectra and fluorescence intensity as those of 5-HT.[1] Therefore, to estimate the amount of α-Me-5HT present after treatment with α-Me-5HTP, it was necessary to subtract the amount of 5-HT found in control animals from the amount of "apparent"* 5-HT found in α-Me-5HTP-treated mice. This method is correct if one assumes that treatment with α-Me-5HTP has no effect on the endogenous 5-HT.

In the study utilizing ^{3}H-α-Me-5HTP, mice were given i.p. 200 mg/kg (50μc) of the drug. The same isolation and determination procedure was used and, in addition, a 1-ml aliquot of the column eluate was counted by placing the aliquot in a scintillation vial containing 15 ml toluene: Trixton X-100 (3 : 1) scintillation fluid[7] followed by counting in a Packard liquid scintillation counter. The amount of ^{3}H-α-Me-5HT present in the brains was calculated by using a specific activity of 2·93 mc/m-mole, a counting efficiency of 6 per cent and a recovery of 66 per cent which was estimated on the basis of the recovery of unlabeled α-Me-5HT added to untreated mouse brain homogenates.

RESULTS

A comparison of the effect of administration of α-Me-5HTP and 5-hydroxytryptophan (5-HTP) on brain serotonin and norepinephrine is presented in Table 1. The greater effectiveness of α-Me-5HTP treatment in causing an increase in "apparent" 5-HT, compared to 5-HTP, is certainly due in part to the lack of metabolism of α-Me-5HT by monoamine oxidase (MAO),[8] whereas the 5-HT synthesized from 5-HTP can be readily metabolized by this enzyme. In addition to the different effects on 5-HT levels, there is also a drastic difference in the two amino acids in their effect on NE

TABLE 1. COMPARATIVE EFFECTS OF α-ME-5HTP ADMINISTRATION ON MOUSE BRAIN AMINE LEVELS*

Drug	Dose (mg/kg)	NE (μg/g ± S.D.)	"Apparent" 5-HT (μg/g ± S.D.)
		0·43 ± 0·02 (3)	0.83 ± 0·09 (3)
α-Me-5HTP	50	0·29 (2)	0·98 (1)
	100	0·19 ± 0·04 (3)	1·15 (2)
	200	0·15 ± 0·02 (3)	1·73 ± 0·18 (3)
5-HTP	50	0·41 ± 0·04 (3)	0·85 ± 0·01 (3)
	100	0·43 (2)	0·98 (2)
	200	0·40 ± 0·01 (3)	1·29 ± 0·06 (3)

*Values in parentheses are number of determinations. Mice were sacrificed 4 hr after i.p. administration of the drug. Two brains were used per assay.

*The term "apparent" 5-HT is used to denote the sum of 5-HT and α-Me-5HT determined, since they are not separated in the isolation and fluorometric procedure.

levels. All doses of α-Me-5HTP (50–200 mg/kg) caused a precipitous drop in NE while 5-HTP treatment exerted no effect on NE levels at doses up to 200 mg/kg.

A time study correlating the rise in brain α-Me-5HT and the decrease in NE is depicted in Fig. 1 The α-Me-5HT level increases rapidly during the first 4 hr and then slowly declines over the next 24–48 hr period. Norepinephrine drops very rapidly, concomitant with the rise in α-Me-5HT, attaining a minimum value in 4–8 hr and complete recovery within 24 hr.

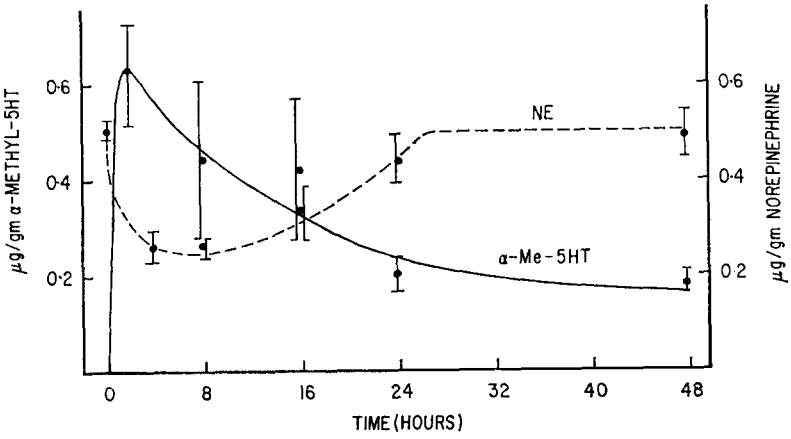

FIG. 1. A time study of the effect of 200 mg/kg of α-Me-5HTP on α-Me-5HT and NE in the mouse brain. Six mice were sacrificed at each of the indicated times, two brains were used per determination. The amount of α-Me-5HT was estimated by subtracting the amount of 5-HT found in control animals from the amount of "apparent" 5-HT found in treated animals. Vertical bars represent S.D.

TABLE 2. EFFECT OF RESERPINE ON "APPARENT" 5-HT AND NE IN THE MOUSE BRAIN

Drug	Drug* schedule (hr)	Dose (mg/kg)	NE (μg/g ± S.D.)	Apparent 5-HT (μg/g ± S.D.)
Control			0·49 ± 0·06	0·49 ± 0·03
α-Me-5HTP	0–8	200	0·29 ± 0·03 (P<0·01)	1·20 ± 0·08 (P<0·01)
Reserpine	0–4	2	0·07 ± 0·02 (P<0·01)	0·15 ± 0·01 (P<0·01)
α-Me-5HTP + reserpine	0–4–8	200–2	0·06 ± 0·02 (P<0·01)	1·19 ± 0·50 (P<0·01)
Reserpine + α-Me-5HTP	0–4–12	2–200	0·07 ± 0·02 (P<0·01)	0·47 ± 0·10 (NS)

*Drug schedule is given in order of drug list and the last time listed is sacrifice time. Two brains were used per determination and three determinations were made per schedule. The *t*-test was used for statistical analysis.

Recent evidence has indicated that pretreatment with α-methyl-m-tyrosine can protect NE and dopamine stores against the action of reserpine.[9] This protection is believed to be afforded by the decarboxylation product of the amino acid, an α-methyl substituted amine.[9] It seemed that α-Me-5HTP treatment should provide a comparable type of protection for 5-HT stores, since it too is decarboxylated to an α-methyl amine. The data presented in Table 2 indicate that indeed this is the case for α-Me-5HTP

pretreatment. Reserpine alone readily causes a diminution in NE and 5-HT stores; α-Me-5HTP (200 mg/kg) alone causes a 40 per cent decrease in NE and an apparent increase in 5-HT of 140 per cent. The administration of α-Me-5HTP (200 mg/kg) prior to a dose of 2 mg/kg of reserpine does protect 5-HT but not NE stores. When mice are pretreated with reserpine, then given α-Me-5HTP, it is seen that the high level of "apparent" 5-HT observed with α-Me-5HTP alone is not attained.

The obvious question to be answered concerning these experiments is whether the administration of α-Me-5HTP and the subsequent formation of α-Me-5HT results in a decrease in endogenous 5-HT and an increase in α-Me-5HT. The same question can be posed for the experiments with reserpine; does reserpine bring about a change in the ratio of 5-HT/α-Me-5HT or does it remain the same. The data presented in Table 3 indicate that after the administration of 200 mg/kg of ^{3}H-α-Me-5HTP there

TABLE 3. COMPARATIVE EFFECT OF RESERPINE ON "APPARENT" 5-HT AND ^{3}H-α-ME-5HT IN THE MOUSE BRAIN

Drugs	Drug schedule (hr)	Dose (mg/kg)	NE (μg/g ± S.D.)	"Apparent" 5-HT (μg/g ± S.D.)	"Apparent" 5-HT less endogenous 5-HT	^{3}H-α-Me-5HT* (μg/g ± S.D.)
			0·31 ± 0·01	0·55 ± 0·03		
^{3}H-α-Me-5HTP	0–8	200	0·15 ± 0·03	1·72 ± 0·17	1·16 ± 0·17	0·92 ± 0·17 (NS)
^{3}H-α-Me-5HTP + reserpine	0–4–8	200–2	0·02 ± 0·01	1·35 ± 0·21	0·80 ± 0·23	0·75 ± 0·16 (NS)

*These values were obtained by using a specific activity of 2·93 mv/m-mole for ^{3}H-α-Me-5HTP. Two brains were used per determination; three determinations were made for controls and five for treated mice. The last time listed is sacrifice time. The *t*-test was used for statistical analysis.

†Obtained by comparing amount of ^{3}H-α-Me-5HT vs. α-Me-5HT, i.e. "apparent" 5-HT less endogenous 5-HT, for each treatment at $P = 0·05$ level.

is no change in endogenous 5-HT and that the increase in "apparent" 5-HT is due to α-Me-5HT. Proof of this is in the fact that the amount of α-Me-5HT determined by isotopic measurements, 0·92 μg/g, is approximately equivalent to the value obtained when one subtracts the amount of 5-HT found in controls from the total amount of "apparent" 5-HT when mice are treated with α-Me-5HTP, 1·17 μg/g. Similar results are obtained after administering reserpine to ^{3}H-α-Me-5HTP-pretreated mice; the amount of ^{3}H-α-Me-5HT is 0·75 μg/g and that obtained fluorometrically, as above, is 0·80 mg/g.

Additional evidence for the prolonged presence of α-Me-5HT in the mouse brain is presented in Table 4. One group of mice was injected with 200 mg/kg of α-Me-5HTP and sacrificed at the designated times, another group of mice was also injected with α-Me-5HTP and 4 hr before they were sacrificed they were given 2·5 mg/kg of reserpine. The levels of "apparent" 5-HT and NE were measured over a 52-hr period. It is apparent that α-Me-5HTP pretreatment protects 5-HT stores against the depleting action of reserpine for at least 24 hr after a single dose of 200 mg/kg, no such protection was afforded NE stores. The protection of 5-HT stores against reserpine by pretreatment with α-Me-5HTP is additional evidence that α-Me-5HT is present in 5-HT containing neurons.

TABLE 4. TIME STUDY OF THE PROTECTIVE EFFECT OF α-ME-5HTP AGAINST RESERPINE*

Drug	Treatment schedule	Dose (mg/kg)	"Apparent" 5-HT (μg/g ± S.D.)	NE (μg/g ± S.D.)
Control			0·46 ± 0·11	0·37 ± 0 04
Reserpine	0–4	2·5	0·18 ± 0·02	0·04 ± 0·03
α-Me-5HTP	0–8	200	1·10 ± 0·21	0·19 ± 0·03
α-Me-5HTP	0–12	200	1·13 ± 0·19	0·27 ± 0·06
α-Me-5HTP	0–20	200	1·27 ± 0·51	0·29 ± 0·04
α-Me-5HTP	0–28	200	0·70 ± 0·06	0·30 ± 0·01
α-Me-5HTP	0–52	200	0·53 ± 0·13	0·35 ± 0·04
α-Me-5HTP + reserpine	0–4–8	200–2·5	0·95 ± 0·031 (NS)	0·04 ± 0·03
α-Me-5HTP + reserpine	0–8–12	200–2·5	1·00 ± 0·15 (NS)	0·05 ± 0·04
α-Me-5HTP + reserpine	0–16–20	200–2·5	0·71 ± 0·12 (NS)	0·05 ± 0·04
α-Me-5HTP + reserpine	0–24–28	200–2·5	0·31 ± 0·16 ($0·01 < P < 0·05$)	0·05 ± 0·04
α-Me-5HTP + reserpine	0–48–52	200–2·5	0·12 ± 0·04 ($P < 0·01$)	0·06 ± 0·01

*Drugs, drug schedule and dosage are presented in order in which administered; sacrifice time is the last time shown. Two mice were used per determination and 3 determinations were made per schedule. Students *t*-test was used for statistical analysis; data from α-Me-5HTP + reserpine treated animals were evaluated with respect to data from similarly treated α-Me-5HTP dosed animals.

TABLE 5. EFFECT OF RESERPINE ON MICE PRETREATED WITH PHENIPRAZINE AND 5-HTP*

Drugs	Drug schedule (hr)	Dose (mg/kg)	5-HT (μg/g ± S.D.)	NE (μg/g ± S.D.)
			0·55 ± 0·04	0·35 ± 0·04
Pheniprazine	0–8	10	0·94 ± 0·12	0·51 ± 0·02
Pheniprazine + reserpine	0–4–8	10–2	1·01 ± 0·04	0·36 ± 0·03
Pheniprazine + 5-HTP	0–1–8	10–30	1·58 ± 0·08	0·63 ± 0·05
Pheniprazine + 5-HTP + reserpine	0–1–4–8	10–30–2	1·38 ± 0·09	0·31 ± 0·07

*Drugs, drug schedule and dosages are presented in order in which administered. Two brains were used per assay and 3 determinations per drug schedule.

The biochemical effect exerted by administration of α-Me-5HTP to mice appears to be similar to that which has been demonstrated using a combined treatment of a MAO inhibitor and 5-HTP. The data in Table 5 demonstrate this effect. Pheniprazine plus 5-HT treatment results in a large increase in 5-HT and this increased level is not affected by reserpine. One obvious difference in this treatment is that NE stores are also protected from reserpine.

DISCUSSION

The results presented here demonstrate that the administration of α-Me-5HTP to mice result in the formation of α-Me5HT in the brain. The identification of α-Me-5HT was further established by using ^{3}H-α-Me-5HTP as the precursor, followed by the subsequent isolation of the amine on an ion-exchange resin. The coupling of this information with the chromatographic data previously presented[1] provides definite proof of the formation of α-Me-5HT from α-Me-5HTP.

A time study of the effects of a single dose of 200 mg/kg of α-Me-5HT and an examination of the protection of 5-HT stores afforded by such treatment, from reserpine-

depleting action, further delineate some of the properties of α-Me-5HTP. Since 5-HT stores partially resist depletion by reserpine 24 hr after a single dose of α-Me-5HTP, this further indicates the prolonged presence of α-Me-5HT in the brain.

Other α-methyl amino acids[9,10] have been shown to protect catecholamine stores against the prolonged action of reserpine. This protection is believed to be provided by the α-methyl amines formed from their respective amino acids.[9] These amines compete with reserpine for the storage sites and thus protect them from reserpine, while serotonin stores are left unprotected.[10] α-Methyl-*m*-tyrosine, in exerting this protective action on the catecholamine stores, decreases amine levels; therefore it appears that the formed amines displace NE and dopamine (DA) from their storage sites for a short time at the expense of protecting these sites against reserpine. In apparent contrast to the protective mechanism of α-methyl-*m*-tyrosine, α-Me-5HTP does not appear to bring about a decrease in endogenous 5-HT while exerting this protective action, but merely increases the amount of "apparent" 5-HT present. It is conceivable that a portion of the α-Me-5HT found in the brain is located in NE storage granules, since the administration of α-Me-5HTP does cause a reduction in the NE level, thus indicating an interaction between NE and α -Me-5HT in NE-containing neurons. However, considerable amounts of α-Me-5HT are in 5HT neurons or protection against reserpine could not be provided.

A consideration of the existing data on serotonin [11] and of the properties of α-methyl substituted biogenic amines[9,12] supports the results obtained here on α-Me-5HT. Serotonin has been shown to have great difficulty in entering and leaving the brain due to the blood–brain barrier. Therefore, serotonin must be formed in the brain by the decarboxylation of 5-HTP and, before leaving the brain, it is primarily deaminated by MAO and the resulting deaminated products can readily exit. After inhibition of MAO, brain serotonin levels increase and the levels can be further increased by the administration of 5-HTP.

On this basis, one would expect the α-methyl analog of serotonin to have difficulty in entering and leaving the brain. Once it is formed in the brain from α-Me-5HTP, it should remain there for a considerable time, since as the amine it cannot readily exit and being an α-substituted analog it is not metabolized by MAO. Finally, it would seem that α-Me-5HT would resist depletion by drugs such as reserpine, since it cannot be deaminated for exit but remains in the brain and competes with reserpine for the binding sites and by doing so it protects them. Such a selective protection of 5-HT stores may prove to be a valuable tool in exploring the role of serotonin in the brain.

Although it has been clearly demonstrated that pretreatment with α-Me-5HTP protects against the depleting action of reserpine, the general depression induced by reserpine, i.e. decrease in motor activity, was not prevented by α-Me-5HTP. This evidence further supports the contention that reserpine-induced depression may be the result of the drug's depleting effects on catecholamines[13,14] and not of its effect on serotonin.[15]

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