

METABOLISM AND BEHAVIOURAL ACTION OF PSYCHOTROPIC TRYPTAMINE HOMOLOGUES

S. SZARA, E. HEARST and F. PUTNEY

Clinical Neuropharmacology Research Center
Saint Elizabeths Hospital, Washington, D.C.

Summary—The possible correlation between metabolic 6-hydroxylation and behavioural action of psychotropic tryptamine homologues has been examined. The unchanged compounds and their 6-hydroxy metabolites are both active in animals but they seem to produce behaviourally different effects: N,N-dialkyltryptamines produce a decrease in motor activity, while the corresponding 6-hydroxy metabolites elicit hyperactivity. The final behavioural result of a particular compound would appear to depend upon the interaction between the effects of the unchanged compound and of the 6-hydroxylated metabolites. The behavioural hyperactivity produced by α -MT, on the other hand, is probably mediated by a mechanism other than 6-hydroxylation.

INTRODUCTION

THE hallucinogenic activity of DL- α -methyltryptamine (α -MT), N,N-dimethyltryptamine (DMT) and N,N-diethyltryptamine (DET) has been well established in man (BOSZORMENYI *et al.*, 1959; MURPHREE *et al.*, 1961; SAI-HALASZ *et al.*, 1958; SZARA, 1956 and 1957). DMT undergoes 6-hydroxylation and demethylation by liver microsomes (SZARA and AXELROD, 1959). Results of previous studies have indicated that 6-hydroxylation of tryptamine derivatives may be a possible pathway for the production of psychoactive metabolites (SZARA and HEARST, 1962).

The present paper amplifies these findings through an examination of possible correlations between the metabolism and the central effects of some alkylated homologues of tryptamine. The rate of 6-hydroxylation was used as the principal measure of metabolic transformation; the behavioural effect on animals was measured in a parallel series of experiments.

METHODS

The N,N-dialkyl homologues were prepared from indole, oxalylchloride and the corresponding di-n-alkylamines, according to the method of SPEETER and ANTHONY (1954). Preparation of 6-hydroxy DMT (6-HDMT) and 6-hydroxy DET (6-HDET) was carried out according to the methods of STOLL *et al.* (1955) and SPEETER and ANTHONY (1954). The following compounds, DL- α -MT and 6-hydroxy-DL- α -methyltryptamine (6-H- α -MT), chlorpromazine hydrochloride and D-amphetamine sulphate were also used.*

The enzymatic 6-hydroxylation of the tryptamine homologues by rat liver microsomes was carried out as described by SZARA and AXELROD (1959). The hydroxylated and/or dealkylated products were extracted into n-butanol at pH 9.0 and reextracted into 0.2 N

*Kindly supplied by Sandoz Pharmaceutical and by Smith, Kline and French Labs.

acetic acid. In order to facilitate the separation of the mono- and dialkyl derivatives, Whatman No. 1 paper, used for the chromatography of extracts, was first sprayed with phosphate buffer (0.1 M; pH 8.5) and then dried. A mixture of methylalcohol, n-amylalcohol, distilled water and benzene (32:15:8:45 v/v) was used as the chromatographic solvent.

The quantitative determination of the 6-hydroxy derivatives in the enzymatic incubation mixture was carried out by the diazotized sulfanilic acid method in the acidic extracts (SZARA and HEARST, 1962). Synthetic 6-HDET was used as a standard* throughout.

In vivo 6-hydroxylation was measured in rat urine (average of 6 animals) and mouse urine (average of 2 groups of 10 animals), which had been collected for 24 hr after intraperitoneal administration of a standard dose of the compounds (3 mg/kg for rats, 5 mg/kg for mice) as a HCl salt in buffered solutions. The urine samples were incubated with bacterial β -glucuronidase (Sigma Co.) for 2 hr at 37 °C in the presence of phosphate buffer (pH 6.0) and a small amount of chloroform. The 6-hydroxy derivatives were extracted and determined by the diazotized sulfanilic acid method (SZARA and HEARST, 1962).

The disruption of the conditioned responses of rats in a "discriminated avoidance" situation (SZARA and HEARST, 1962) served as one measure of the behavioural action of the drugs. Every determination of the behavioural threshold dose (the minimum dose which brought about a definite impairment of avoidance behaviour) was based on at least 3 animals. All behavioural threshold doses (BTD) were secured only after the subjects had already achieved stable and reliable day-to-day performance in the absence of the drug.

The action of the compounds on the motor activity of mice was measured in a photocell activity cage (KINNARD and CARR, 1958). The activity of drug-treated groups of 4 animals was compared with the activity of a simultaneously tested placebo-treated group. The results are presented as the average of 90-min counts, calculated from at least three groups of 4 animals ($\pm$ standard deviation).

RESULTS

Figure 1 shows the photograph of a typical one-dimensional chromatogram for the enzymatically formed basic metabolites of the four lower homologues. *M* denotes the starting point of the extracts obtained from incubated DMT; *Ta*_s is a tryptamine standard and *M*_s is DMT standard. *E*, *P*, *B* are the corresponding starting points of DET, N,N-dipropyltryptamine (DPT) and N,N-dibutyltryptamine (DBT) respectively. The adjacent *E*_s, *P*_s and *B*_s are the corresponding standards. The purple spots (obtained with Ehrlich's *p*-dimethylaminobenzaldehyde spray) in the chromatogram of *M* are DMT, monomethyltryptamine and tryptamine (in decreasing order of *R*_f values). Very close to the tryptamine spot, but distinctly blue, is the spot of 6-HDMT. The chromatogram of *E* shows unchanged DET, monoethyltryptamine and the blue 6-HDET. The chromatogram of *P* shows DPT, monopropyltryptamine and the blue 6-hydroxy DPT; the latter's *R*_f value is between those of the former two compounds. *B* shows the presence of unchanged DBT, monobutyltryptamine and a small amount of 6-HDBT, which is slightly overlapping with the tail of the former compounds. The higher homologues, diamyltryptamine and dihexyltryptamine (DHT) were not included on this figure because they showed only minute amounts of the metabolites on paper.

*The variation in the recovery of the different 6-hydroxy homologues in these tests was within acceptable limits ($\pm$ 10 per cent of the values obtained with 6-HDET).

Enzymatic dealkylation and 6-hydroxylation of dialkyl-tryptamine derivatives (Basic extracts: Whatman No. 1, buffered at pH 8.5; MeOH: AmOH: H₂O: Bz = 32:15:845; Ehrlich's spray)

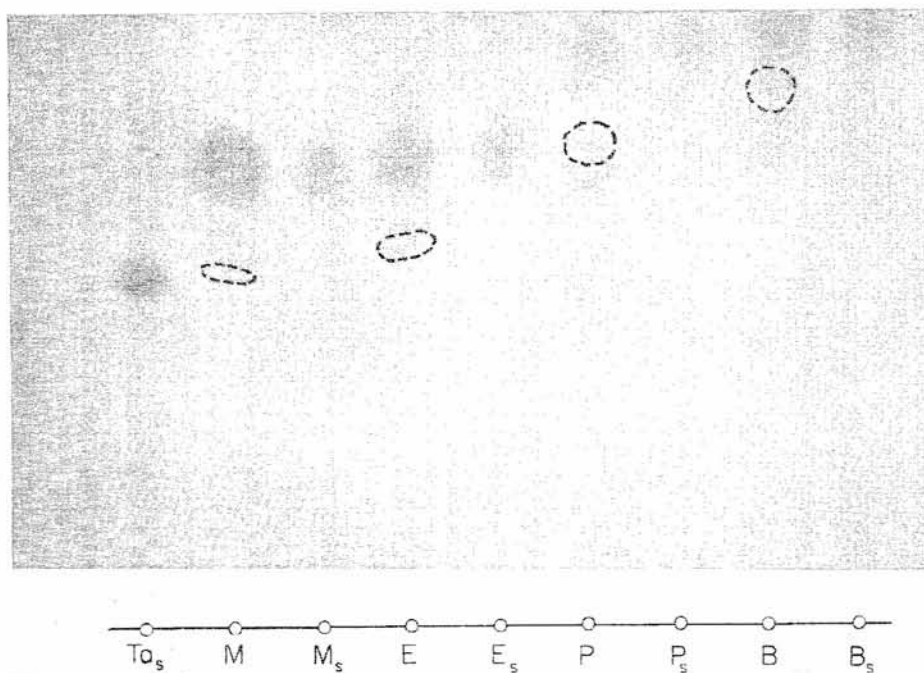


FIG. 1. Photograph of a typical one-dimensional chromatogram for the enzymatically formed basic metabolites of N,N-dimethyltryptamine (DMT), N,N-diethyltryptamine (DET), N,N-dipropyltryptamine (DPT) and N,N-dibutyltryptamine (DBT). The spots marked with broken lines are blue with Ehrlich's reagent while the unmarked ones are purple.

TABLE 1. RELATIVE RATE OF 6-HYDROXYLATION OF INDOLE DERIVATIVES
BY RAT LIVER MICROSOMES
(DET=100)

Substrate (2 μ M/ml)	%
Tryptamine (T)	15
Dimethyl-T	32
Diethyl-T	100
Dipropyl-T	81
Dibutyl-T	19
Diamyl-T	3
Dihexyl-T	0.5
α -Methyl-T (d.l-)	22
L.Tryptophan	0

Table 1 gives relative rates of 6-hydroxylation for the various substrates as measured after 1 hr of incubation and expressed as per cent of O.D. values obtained with DET. Under these conditions, DET seems to be the best substrate among the various compounds tested. The lower and higher homologues gave smaller readings than DET. The higher homologues proved extremely poor substrates for the microsomal 6-hydroxylation at the substrate concentration tested (2 μ M/ml).

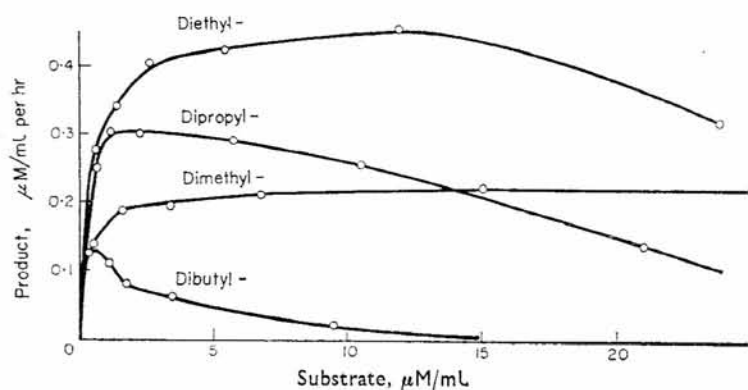


FIG. 2. Inhibition of 6-hydroxylation of the dialkyl-homologues of tryptamine by substrate appears to be a function of chemical structure.

Figure 2 indicates that amount of inhibition of hydroxylation by substrate appears to be a function of chemical structure. The 6-hydroxylation of DMT follows the Michaelis-Menten rule, but homologues higher than DMT (especially DBT) show increasingly more pronounced inhibition by substrate. DHT is hydroxylated to such a small extent that it could not be shown on the same scale.

Table 2 shows that the learned behaviour of rats is differentially disrupted by these drugs. Behavioural threshold doses (BTD) and potency are listed. The metabolite, 6-HDET, is about twice as potent as the parent drug, DET. DBT is practically inactive up to 20 mg/kg doses. Interestingly enough, DHT is active in relatively low doses. However, the animals given DHT showed less violent reactions to shock than the lower-homologue treated

TABLE 2. BEHAVIOURAL POTENCY OF INDOLE DERIVATIVES IN RATS ON CONDITIONED SHOCK AVOIDANCE SCHEDULE

Compound	Behavioural threshold dose (BTD) mg/kg	Potency 1/BTD
DL- α -methyltryptamine	2.5	0.40
N,N-diethyltryptamine (DET)	11.0	0.09
6-Hydroxy-DET	5.5	0.18
N,N-dipropyltryptamine (DPT)	18.0	0.05
N,N-dibutyltryptamine (DBT)	>20.0	<0.05
N,N-dihexyltryptamine (DHT)	3.4	0.29
3-Indoleacetic acid	>20.0	<0.05

subjects; in addition, animals given supra-threshold doses of the lower homologues often displayed ataxia and disorientation and salivated profusely; reactions not observed under effective doses of DHT. Thus, DHT appeared to act on the animals in a way different from the rest of the listed compounds, but the behavioural test used was not specific enough to show these differences quantitatively, since all compounds brought about a deterioration of avoidance performance.

TABLE 3. EFFECT OF TRYPTAMINE DERIVATIVES ON SPONTANEOUS ACTIVITY OF MICE

Compound	Dose mg/kg i.p.	Average count* per 90 min	Activity ratio
Placebo	—	491 $\pm$ 95	1.00
DL-Amphetamine	2.5	820 $\pm$ 166	1.67 **
	5.0	3056 $\pm$ 250	6.23 **
Chlorpromazine	5.0	371 $\pm$ 140	0.76
	10.0	50 $\pm$ 30	0.10 **
DL- α -Methyltryptamine (α -MT)	10.0	961 $\pm$ 205	1.96 **
	20.0	2290 $\pm$ 166	4.67 **
DL-6-Hydroxy- α -MT	10.0	757 $\pm$ 176	1.54 **
N,N-dimethyltryptamine (DMT)	5.0	630 $\pm$ 182	1.28
	10.0	453 $\pm$ 82	0.92
	20.0	305 $\pm$ 138	0.62 **
6-Hydroxy-DMT	10.0	1011 $\pm$ 160	2.01 **
N,N-diethyltryptamine (DET)	2.5	538 $\pm$ 47	1.10
	5.0	741 $\pm$ 162	1.51 **
	10.0	685 $\pm$ 140	1.40 **
	20.0	282 $\pm$ 126	0.57 **
6-Hydroxy-DET	5.0	876 $\pm$ 105	1.78 **
	10.0	582 $\pm$ 46	1.18
N,N-dipropyltryptamine	10.0	763 $\pm$ 182	1.56 **
N,N-dibutyltryptamine	10.0	375 $\pm$ 78	0.76
N,N-dihexyltryptamine	5.0	221 $\pm$ 43	0.45 **
	10.0	238 $\pm$ 175	0.48 **
	20.0	133 $\pm$ 65	0.27 **

*Photocell activity cage, groups of 4 mice, average of at least 3 groups.

**Statistically significant at the $p=0.05$ level.

Measurement of the activity of mice in the photocell activity cage helped to distinguish between the effect of DHT and that of the lower homologues. Table 3 gives the average of 90 min activity counts, as well as the activity ratios calculated by dividing the experimental values by the placebo values. An activity ratio above 1.0 means hyperactivity (as in the case of DL-amphetamine) and a ratio below 1.0 means hypoactivity (as for Chlorpromazine).

The lower homologues (DMT, DET and DPT) in non-toxic doses produce hyperactivity, as do their 6-hydroxy metabolites. DBT and DHT, on the other hand, produce hypoactivity.

6-H- α -MT is slightly less active than α -MT in the same dose, but 6-HDMT and 6-HDET are more active than corresponding doses of the parent compounds. Decreases in the activity ratio with higher doses of DMT and DET appeared to be due to ataxia as part of the hyperexcitation, rather than to tranquillization.

Figure 3 summarizes the relationships between *in vivo* 6-hydroxylation and the behavioural action of these compounds. The lower dialkylated homologues (DMT, DET, DPT and DBT) show a definite correlation between the rate of 6-hydroxylation and behavioural activity. Compounds with low 6-hydroxylation values generally have low potency in behavioural tests, while compounds with higher 6-hydroxylation values possess high potency in the same tests. DHT shows very low 6-hydroxylation and a qualitatively different behavioural activity.

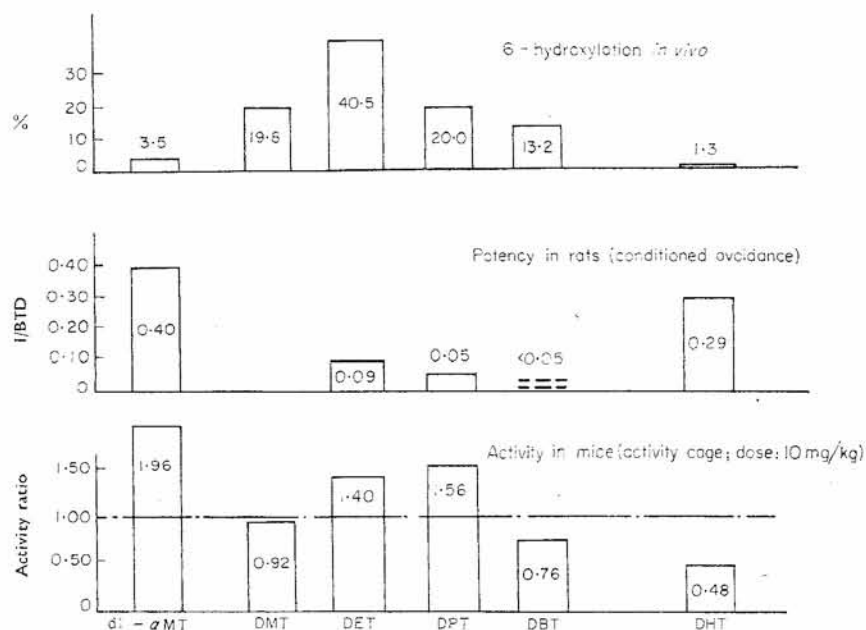


FIG. 3. Summary of the relationships between *in vivo* 6-hydroxylation and the behaviour of alkylated tryptamine homologues.

α -MT stands out of the series not only chemically (it is a primary amine), but also behaviourally. It produced hyperactivity in mice and there was a lag period (30–45 min.) between injection and behavioural changes; a similar lag period before response was observed

in human subjects (MURPHREE, DIPPY, JENNEY and PFEIFFER, 1961). In addition, α -MT showed a low rate of *in vivo* 6-hydroxylation and the 6-hydroxy metabolite possessed relatively low behavioural potency.

DISCUSSION

We are tempted to interpret the behavioural action of dialkylated tryptamine derivatives as a result of the separate actions of the parent compound and the 6-hydroxy metabolite. From a strictly behavioural point of view, and within the limits of the two tests here employed, the unchanged compounds seem to produce a calming, tranquillizing effect while the 6-hydroxy metabolites seem to elicit a behavioural effect more like excitation. The result of these interactions is primarily dependent upon the *in vivo* rate of 6-hydroxylation of a particular member of the series.

In view of the biochemical and behavioural differences between α -MT and the dialkylated compounds it seems probable that the hyperactivity produced by α -MT is mediated by a mechanism other than via 6-hydroxylation.

It would be worthwhile to investigate the relationships between the metabolism of these compounds and their hallucinogenic activity in man. We have presented some preliminary data (SZARA and ROCKLAND, *in press*) which agree with our hypothesis that 6-hydroxylation may be intimately involved in the production of autonomic changes, and anxiety and perceptual disturbances seen following the administration of DET (1 mg/kg of body weight to normal volunteers). Nevertheless, more detailed and definitive studies will be needed to clarify these possible relationships.

Résumé—On a étudié les corrélations éventuelles entre la 6-hydroxylation, métabolique et l'influence des homologues psychotropes de la tryptamine sur le comportement. Les drogues non altérées et leurs 6-hydroxy-métabolites exercent une action *in vivo*, mais leurs influences sur le comportement des animaux diffèrent l'une de l'autre. Ainsi, les N,N-dialcoyltryptamines diminuent l'activité motrice alors que les métabolites 6-hydroxylés déclenchent une hyperactivité. En ce qui concerne le comportement, le résultat final d'une combinaison déterminée dépend de l'interaction entre les effets de la drogue non altérée et des 6-hydroxy-métabolites. D'autre part, l'hyperactivité motrice provoquée par l' α -MT semble pouvoir être attribuée à un mécanisme autre qu'une 6-hydroxylation.

Zusammenfassung—Es wurden die möglichen Wechselbeziehungen zwischen einer 6-Hydroxylierung im Stoffwechsel und einer Wirkung psychotroper Tryptamin-Homologe auf das Verhalten von Tieren untersucht. Die unveränderten Verbindungen und ihre 6-Hydroxy-Stoffwechselprodukte sind beide im Tierversuch wirksam, aber sie scheinen doch auf das Verhalten unterschiedlich einzuwirken: N,N-dialkyltryptamine ergeben ein Nachlassen der motorischen Aktivität, wogegen die entsprechenden 6-Hydroxy-Stoffwechselprodukte eine Hyperaktivität herbeiführen. Der endgültige Einfluss einer bestimmten Verbindung auf das Verhalten ist wohl abhängig von der Wechselwirkung zwischen den Effekten der unveränderten Verbindung und den 6-hydroxylierten Stoffwechselprodukten. Die verhaltensmässige Überaktivität, die durch α -MT ausgelöst wird, wird dagegen wahrscheinlich durch einen anderen Mechanismus als eine 6-Hydroxylation bewirkt.

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Author's address—S. SZARA, Section on Psychopharmacology, Clinical Neuropharmacology Research Center, National Institute of Mental Health, Saint Elizabeths Hospital, WASHINGTON, D.C., U.S.A.

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