Biochemical Pharmacology, Vol. 25, pp. 1521–1527. Pergamon Press, 1976. Printed in Great Britain.

EFFECTS OF IPRONIAZID AND TRANYLCYPROMINE ON THE HALF-LIFE OF *N,N*-DIMETHYLTRYPTAMINE IN RAT BRAIN AND LIVER*

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(*Received* 22 *November* 1974; *accepted* 5 *November* 1975)

Abstract—The time course of *N,N*-dimethyltryptamine (DMT) levels in brain and liver of adult male Holtzman rats was determined with and without pretreatment with the monoamine oxidase inhibitors iproniazid (32 mg/kg) and tranylcypromine (10 mg/kg) given i.p. DMT was given in doses of 1.0, 3.2 and 10.0 mg/kg i.p. DMT was assayed spectrophotofluorometrically and in some instances by a radioisotopic method. The results show that the half-life of DMT varied with different doses in the brain but not in the liver. Pretreatment with the monoamine oxidase inhibitors prolonged the total period during which DMT was found. A linear relationship exists between the mean time required to reach minimal DMT concentrations and the mean duration of suppression of FR_4 barpressing behavior in rats trained for a milk reward. The results suggest a direct involvement of DMT in inducing behavioral toxicity.

N,N-dimethyltryptamine (DMT) is one of the hallucinogenic indolealkylamines [1–3]. Biosynthetic pathways exist for the potential synthesis of DMT *in vivo* [4–8]. This hallucinogen has been implicated in the pathogenesis of schizophrenia [9, 10] although the findings are controversial [11, 12]. DMT is also abused as a hallucinogen [13, 14].

DMT is known to produce abnormal behavior in animals [15–21]. Much of the speculation on the mode of action of DMT is centered on its interaction with 5-hydroxytryptamine (5-HT) [22, 23]. Nevertheless, the time course of the increase in brain 5-HT [23] seems in disagreement with the duration of its behavioral deficits [24]. Many explanations can be offered for this discrepancy. Nevertheless, the direct involvement of DMT in inducing behavioral deficits is the most parsimonious. DMT can readily be detected in the rat brain after systemic injections [25]. It is known to disappear very rapidly from biological tissues. However, to our knowledge no attempt has been made to determine its half-life in various tissues.

The purpose of this study is to determine in rats the rate of disappearance of DMT and to determine whether DMT-induced behavioral deficits are correlated with its concentration in the brain. Sai-Halász [26] reported that the hallucinatory response to DMT in humans was diminished upon pretreatment with iproniazid. However, iproniazid prolonged and potentiated the toxic effects of DMT in animals using various behavioral endpoints [24].† Therefore, the effects of both iproniazid and the now more widely used monoamine oxidase (MAO) inhibitor tranylcypromine were studied to determine if such behavioral potentiation in rats was a direct result of an elevated tissue DMT level.

METHODS

Design of experiments. Male Holtzman rats, at least 90 days old, weighing 400–450 g, were used. Control animals received i.p. either 0.9% NaCl (1–2 ml/kg) 2 and 16 hr, iproniazid (32 mg/kg) 16 hr, or tranylcypromine (10 mg/kg) 2 hr prior to guillotining. Experimental rats received either 0.9% NaCl (2 ml/kg) 2 and 16 hr, iproniazid (32 mg/2 ml/kg) 16 hr, or tranylcypromine (10 mg/kg) 2 hr prior to the administration of DMT in doses of 1–10 mg/kg. In some experiments, ^{14}C-labeled DMT (side chain-[1-^{14}C], purchased from New England Nuclear) in a dose of 3.2 mg/kg (sp. act. 0.94 μCi/mg) of compound was given. Each group consisted of five to ten animals. They were killed at different time intervals after DMT. After decapitation, the brain and liver were immediately removed and homogenized in 4 ml of ice-cold 1 N HCl. All data were compared with the controls run during the same experiment. Group comparison Student's *t*-tests [27] were used to determine statistical significance. Groups of rats were trained to barpress on a fixed ratio of four barpresses to one milk reward (FR_4) schedule [24]. Trained rats received either 3.2 or 10 mg/kg of DMT i.p. The rats were sacrificed as soon as they resumed normal barpressing behavior. DMT content in brain was then analyzed.

DMT assay. Homogenates were centrifuged at 9000 *g* to give cleaner supernatants, which were separated from precipitates and collected. The precipitates were each washed twice with 1.5 ml of 0.1 N HCl, shaken by a Vortex mixer, centrifuged, and the supernatants collected. All the acidic supernatants were then combined and used for subsequent assay. The

* Supported in part by a grant from the State of Michigan for schizophrenia research. Part of this material was presented at the ASPET Meeting in Montreal, August 1974 (*Pharmacologist* **16**, 237, 1974).

† M. Lutz and E. F. Domino, unpublished data.

separation of supernatants is essential to give a clean extraction with organic solvent. To separate DMT from other metabolites, the combined acidic supernatants were made basic with 2 ml of 10 N NaOH and extracted with 30 ml of a mixture of ethyl acetate and toluene (1:1 by volume). An aliquot of 25 ml of the organic solvent was removed and shaken with 2 ml of 0.1 N HCl. DMT was determined fluorometrically (285 nm/350 nm, uncorrected) by the method of Cohen and Vogel [25] in the Farrand spectrofluorometer, after the adjustment of the pH to 8–9 with 0.1 M sodium borate buffer.

In those experiments in which [^{14}C]DMT was given, the rats were sacrificed by decapitation 10 min later. Unchanged [^{14}C]DMT was extracted according to the procedure described for the fluorometric determination. To count total radioactivity of unchanged [^{14}C]DMT, an aliquot of 1.5 ml of 0.1 N HCl was basified with 100 μl of 10 N NaOH and extracted with 3 ml of the mixture of ethyl acetate and toluene. An aliquot of 2 ml of the organic phase was mixed with 10 ml Aquafluor and counted in a liquid scintillation counter. The result obtained from this method was compared with that obtained from the fluorometric method.

Qualitative presence of DMT in tissue extracts was demonstrated by thin-layer chromatography (t.l.c.) and radioscanning. The ethyl acetate and toluene phase of tissue extracts from rats receiving [^{14}C]DMT was evaporated to dryness under a nitrogen stream. The residue was redissolved in 100 μl ethyl acetate. Aliquots of known radioactivity were spotted on a Silica gel GF plate (Analtech, Inc., Newark, Del.) and developed in the following solvent systems: (1) methanol–conc. ammonium hydroxide (300:45); the R_f of tryptamine (T) was 0.32, of *N*-methyltryptamine (NMT) 0.18, and of DMT 0.42; (2) isopropanol–10% ammonium hydroxide–water (200:10:20); the R_f value of T was 0.15, of NMT 0.05 and of DMT 0.23; and (3) methanol–tetrahydrofuran–formic acid (50:50:1); the R_f value of T was 0.5, of NMT 0.32 and of DMT 0.25. Radioscanning of brain and liver extracts showed only one spot with detectable radioactivity and with an R_f value of that of known DMT. T.l.c. plates were then scored and each segment was removed. Radioactivity in each segment was counted in a liquid scintillation counter. The majority (97–98 per cent) of radioactivity appeared in the segment with an R_f of that of DMT in contrast to the radioactivity detected for that of T and NMT.

Chemical separation of tryptamine and N-*methyltryptamine from DMT*. Samples containing indolealkylamines (T, NMT or DMT, radioactive material if available) in 30 ml of ethyl acetate and toluene were cooled, and 0.5 ml trifluoroacetic anhydride (TFAA) was added. The mixtures were then set in an ice bath for 0.5 hr. Excess TFAA was then destroyed with 7 ml of ice-cold H_2O, basified with 3 ml of 10 N NaOH, vortexed, and centrifuged. Control samples which did not receive TFAA were washed with 10 ml of a premixed combination of ice-cold H_2O–10 N NaOH–trifluoroacetic anhydride (7 ml:3 ml:0.5 ml). An aliquot of 25 ml of the ethyl acetate and toluene phase was then transferred to another tube, extracted with 3 ml of 0.1 N HCl, vortexed, and centrifuged. An aliquot of 2 ml of 0.1 N HCl extract was neutralized with 1 ml of 10 N NaOH with cooling. The basic solution was then extracted with 3 ml of a mixture of ethyl acetate and toluene. To count total radioactivity, 2 ml of the organic phase was mixed with 10 ml Aquafluor and was counted in a liquid scintillation counter. In the case of NMT, where radioactive material was not available, the recovery and yield of its reaction with TFAA were determined by a fluorometric method: an aliquot of 1 ml of 0.1 N HCl was mixed with 1 ml of 0.1 M sodium borate buffer and fluorescence was read at an excitation wavelength of 285 nm and an emission wavelength of 350 nm (uncorrected).

Calculations. The biological half-life of DMT was determined according to the equation $y = ae^{x/b}$, where y is the original datum of DMT concentration in μg/g of tissue; x is time in min from i.p. injection of DMT to sacrifice; and a represents the concentration at zero time. Half-life ($t/2$) was computed by the equation $(t/2) = -b \ln 2$ and estimated according to the nonlinear regression analysis of Gauss [28]. Exponentials were fitted directly by solving the "normal equations of curve fitting" with reciprocals of the observed variance estimates as weights. The parameters (initial concentration and half-life) were adjusted iteratively until both were stable to 0.01 per cent. Confidence limits were based on a small error analysis using the product-variance matrix. After i.p. injection of DMT, its half-life was calculated from data measured after peak tissue DMT levels were reached, for absorption is presumably complete at that time.

RESULTS

As described in Methods, a mixture of ethyl acetate and toluene was used for the extraction of DMT from a basic tissue extract to ensure consistent and better recovery (80–90 per cent). Extraction with toluene alone, especially in the presence of tissue, gave variable results of recovery. The sensitivity of the assay is about 0.05 μg/ml of sample, which is in agreement with Cohen and Vogel [25].

Fluorescence of extracts from 0.9% NaCl-pretreated animals was negligible. Extracts of tissue from iproniazid-pretreated control animals gave some fluorescence. This fluorescence could possibly come from endogenous T resulting from the pretreatment of iproniazid rather than DMT. However, when it was calculated as if it were DMT, then the mean $\pm$ S. E. fluorescence intensity was equal to 0.50 ± 0.15 μg/g of brain and 1.16 ± 0.14 μg/g of liver DMT. Tranylcypromine-pretreated control rats also gave some fluorescence. The mean $\pm$ S. E. fluorescence intensity was equal to 0.24 ± 0.11 μg/g of brain and 0.45 ± 0.07 μg/g of liver DMT.

Table 1 lists DMT concentrations in rat tissues (control values subtracted) 10 min after administration of the drug (3.2 mg/kg) as determined by various methods. No significant difference in DMT levels could be detected between the two groups of five to six rats.

Thin-layer chromatography of the concentrated ethyl acetate and toluene phase of tissue extracts indicated the presence of DMT with an R_f value of 0.42 (MeOH and NH_4OH), 0.19 (isopropanol, 10%

Table 1. Comparison of DMT concentrations (μg/g) in rat tissues (3.2 mg/kg at 10 min)

	Fluorometric method	Radioisotopic method*	P
Brain	1.60 ± 0.11	1.55 ± 0.18	>0.82
Liver	1.56 ± 0.25	1.54 ± 0.32	>0.96

* Each rat received 3 μCi/kg, i.p. The mean ± S. E. of five to six rats is given for each method of assay.

Table 2. Per cent recovery of indolealkylamines after their reaction with trifluoroacetic anhydride

	Reaction with TFAA		
Samples	(0 ml TFAA)	(0.5 ml TFAA)	P
DMT*	94.5 ± 1.4 (8)†	83.1 ± 1.7 (8)	<0.05
NMT‡	92.5 ± 2.3 (4)	0.0 (6)§	<0.001
T	79.5 ± 3.2 (5)	2.7 ± 0.6 (5)	<0.001
Brain	94.1 ± 1.2¶ (6)	88.3 ± 3.4 (6)	NS
extracts‖	99.6 ± 1.3¶ (6)	106.4 ± 4.1¶ (6)	NS
Liver	99.2 ± 1.0¶ (6)	87.4 ± 2.2 (7)	NS
extracts‖	105.0 ± 1.1¶ (6)	103.7 ± 2.6¶ (7)	NS

* The purity of DMT, as determined by t.l.c. and quantified by liquid scintillation counting, was 99 per cent.

† Number of determinations is in parentheses. Mean per cent ± S. E. is given.

‡ Recovery of NMT was determined by fluorometric method, since its radioactive starting material was not available.

§ Fluorescence readings were negligible. This indicated that the removal of NMT was nearly complete.

‖ Tissue extracts were obtained from rats given 10 mg/kg of DMT (sp. act. 0.81 μCi/mg), sacrificed 10 min after administration.

¶ Results were corrected for recoveries. Reaction of tissue extracts with or without TFAA did not change the recovery significantly.

NH_4OH and H_2O) and 0.25 (MeOH, tetrahydrofuran and formic acid), which were in agreement with that of pure DMT. Radioscanning indicated one spot with detectable radioactivity with the R_f value of DMT.

Figure 1 is a flow diagram of the separation of T and NMT from DMT by reaction with TFAA. Recovery results are shown in Table 2. TFAA treatment removed 98–100 per cent of the T and NMT.

The ethyl acetate and toluene phase of the tissue extracts containing radioactive DMT or possibly NMT and T was equally divided into several portions. Half of the samples were treated with TFAA and their recoveries of total radioactivity were compared with those samples which were not treated with TFAA. The data are given in Table 2.

Figure 2 illustrates the concentration of DMT in rat brain and liver as a function of time after its i.p. administration. The peak concentration of DMT was reached around 10 min after injection in the 0.9% NaCl-pretreated animals. After a dose of 1 mg/kg of DMT, it was possible to detect some DMT in brain (0.24 ± 0.07 μg/g) and liver (0.42 ± 0.13 μg/g) 3 min after administration. The mean ±S.E. half-life of DMT in rat liver was estimated to be 5.9 ± 1.1 min after a dose of 3.2 mg/kg and 7.4 ± 0.7 min after a dose of 10 mg/kg. There was a slight difference in the half-life of DMT at these two given doses, but this was not statistically significant (P > 0.05, Fig. 2). Surprisingly different half-lives of DMT in the brain were noted after different doses. The mean ± S. E. half-life

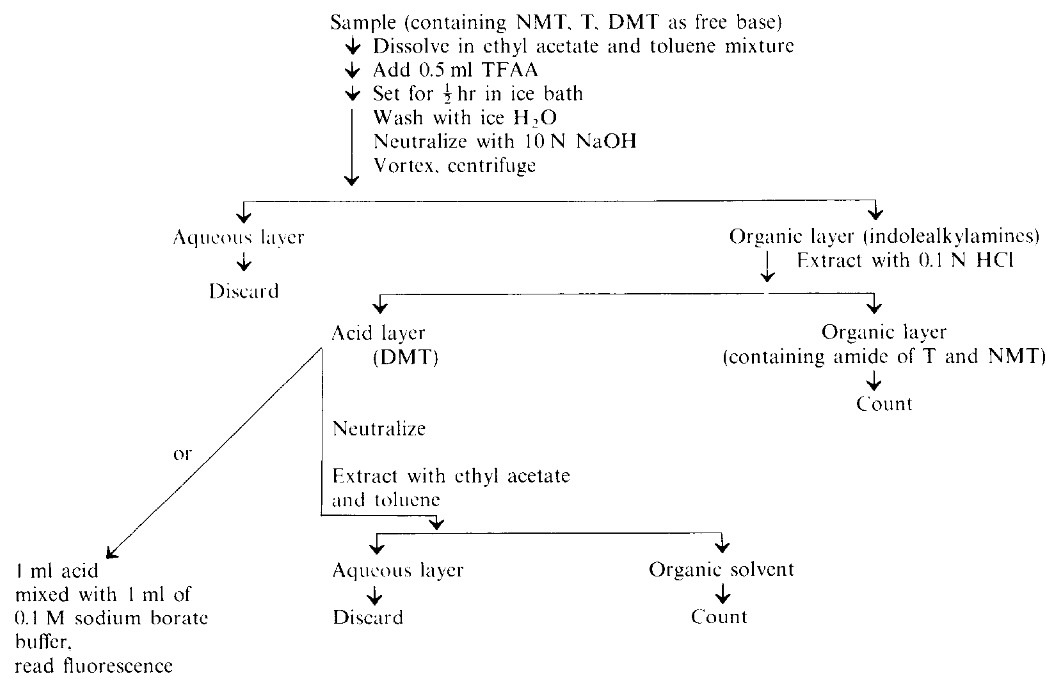

Fig. 1. Flow chart illustrating the separation of T, NMT and DMT with TFAA.

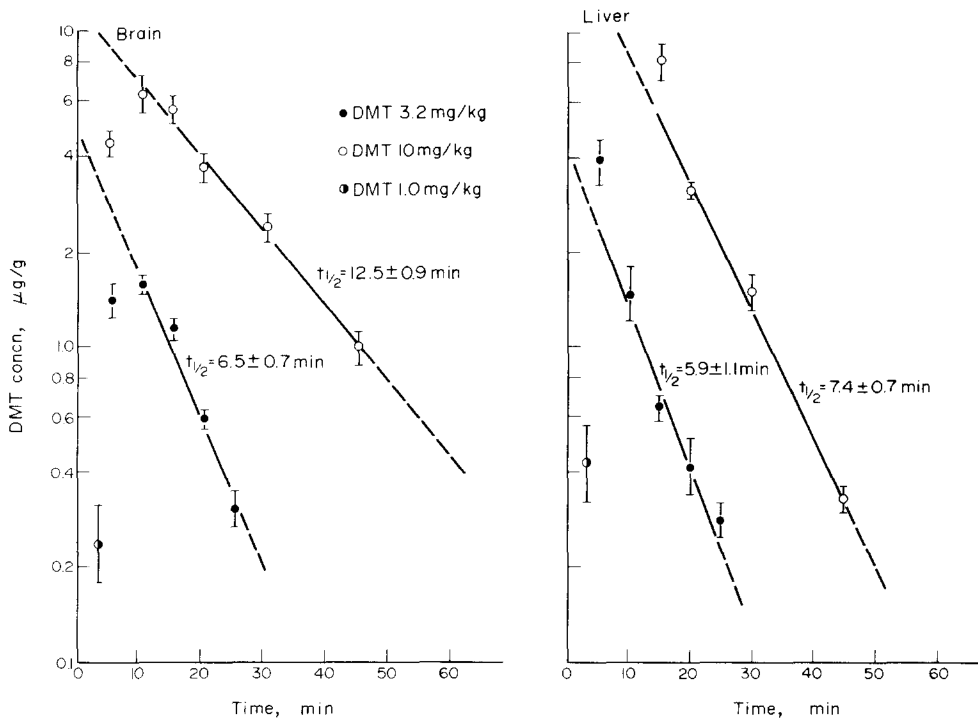

Fig. 2. Half-life of DMT in rat brain and liver. Each point represents the mean DMT concentration of five to ten animals. Each group of animals was sacrificed by guillotine at various times after DMT injection i.p. as illustrated. Note that the half-life of DMT differs in the brain when given in doses of 3.2 vs 10 mg/kg i.p. On the other hand, the half-life of DMT in the liver is the same irrespective of dose.

of DMT in rat brain was estimated to be 6.5 ± 0.7 min when given in a dose of 3.2 mg/kg and 12.5 ± 0.9 min when given in a dose of 10 mg/kg (Fig. 2). The difference is highly statistically significant ($P < 0.001$). If the rats were pretreated with 32 mg/kg of iproniazid i.p., the mean ± S. E. half-life of DMT was estimated to be 30.6 ± 1.7 min in brain and 27.6 ± 0.7 min in liver (Fig. 3). The half-life of DMT in iproniazid-pretreated animals was greatly increased for both brain and liver from that of 0.9% NaCl-pretreated animals ($P < 0.001$). The half-life of DMT (10 mg/kg) in tranylcypromine-pretreated animals was 38.5 ± 2.5 min in brain and 39.7 ± 2.7 min in liver (Fig. 3). Tranylcypromine also greatly increased the half-life of DMT in both brain and liver ($P < 0.001$).

Correlation between tissue DMT level and FR_4 barpressing behavior in male Holtzman rats. It has been previously observed by Kovacic and Domino [24] that rats trained to barpress for milk reward on an FR_4 schedule ceased to press for a mean period of 23 min after 3.2 mg/kg of DMT, 46 min after 10 mg/kg of DMT, and 190 min in rats pretreated with iproniazid (32 mg/kg) and then given 10 mg/kg of DMT 16 hr later. Little or no behavioral disruption could be observed after 1 mg/kg of DMT. As is evident in Fig. 2, the actual brain DMT level is about 0.41 μg/g 23 min after 3.2 mg/kg of DMT. The brain DMT level is about 0.95 μg/g 46 min after 10 mg/kg of DMT. Therefore, the minimal brain DMT level for suppression of barpressing behavior is between 0.41 and 0.95 μg/g.

To verify the estimated threshold DMT level for disruption of barpressing behavior, groups of trained rats were given either 3.2 or 10 mg/kg of DMT i.p. The rats were sacrificed by decapitation as soon as they resumed normal barpressing behavior. The mean ± S. E. in their brains was analyzed and found to be 0.48 ± 0.09 μg/g of tissue after a dose of 3.2 mg/kg and 0.90 ± 0.13 μg/g after a dose of 10 mg/kg of DMT. These data are in agreement with the levels estimated through the time courses of clearance of brain DMT (Fig. 2). A slight but significant difference on the threshold levels for the disruption of barpressing behavior was noted upon the administration of different doses (3.2 and 10 mg/kg) of DMT ($P < 0.03$). The reason for this discrepancy is unknown.

Figure 4 illustrates the correlation between the mean time required to reach minimal DMT concentration in tissues (0.24 μg/g of brain and 0.41 μg/g of liver) and the mean duration of suppression of FR_4 barpressing behavior. The values used are the DMT concentrations in brain and liver after 1 mg/kg of DMT i.p. There is a linear relationship between the mean time required to reach the minimal DMT concentrations in tissues and the mean duration of suppression of FR_4 barpressing behavior with a correlation coefficient of 0.99 for brain ($P < 0.02$) and 1.00 for liver ($P < 0.001$). Brain and liver DMT levels were highly correlated ($r = 0.97$, Fig. 5).

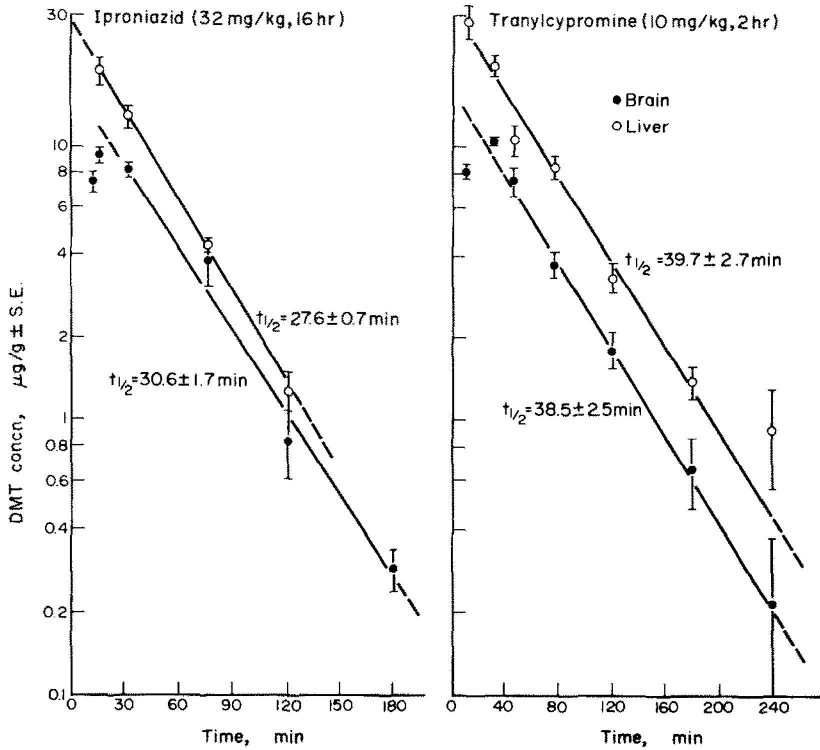

Fig. 3. Effects of iproniazid and tranylcypromine on the half-life of DMT in rat brain and liver. Note the difference in time scales between iproniazid and tranylcypromine pretreatment. After both MAO inhibitors, the half-lives of DMT in brain and liver are now similar. However, the half-lives of DMT for the tranylcypromine-pretreated animals are significantly longer than those of animals given iproniazid ($P < 0.05$). Iproniazid was given 16 hr and tranylcypromine 2 hr before DMT (10 mg/kg).

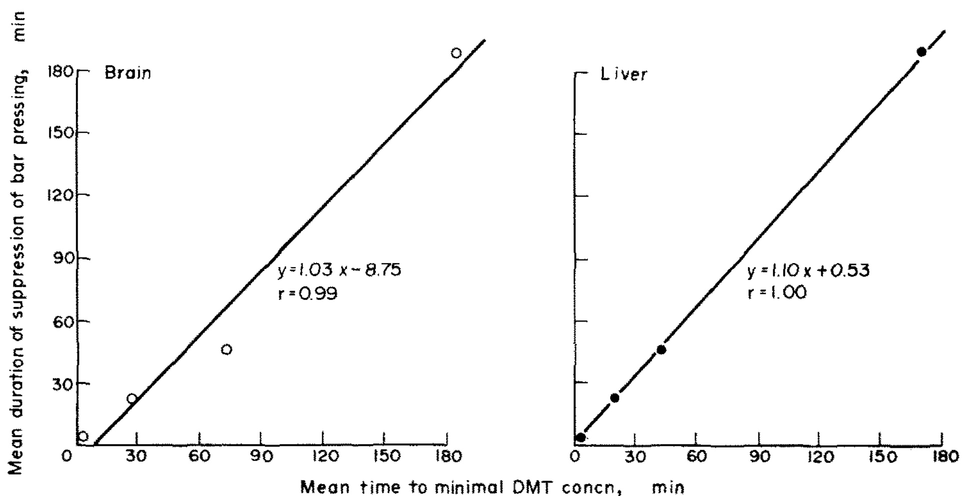

Fig. 4. Correlation between mean time required to reach minimal DMT concentrations in rat brain and liver and the duration of suppression of FR_4 barpressing behavior. In view of the fact that 1.0 mg/kg i.p. of DMT produced little or no behavioral deficits and achieved a concentration of 0.24 µg/g of brain and 0.41 µg/g of liver 3 min after injection, these concentrations were chosen for these tissues. The time for DMT at the different doses to reach these levels was obtained from the half-life data described in Fig. 2. These times were correlated with the duration of suppression of FR_4 barpressing behavior at the different doses from the data of Kovacic and Domino [24].

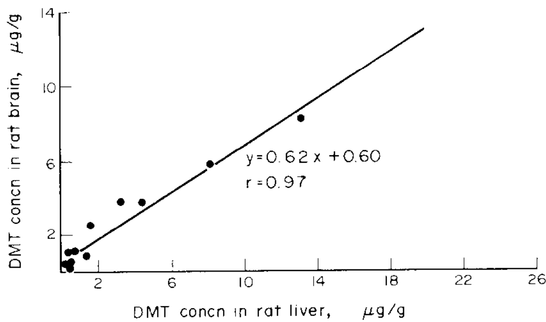

Fig. 5. Correlation of rat brain and liver DMT levels. The concentrations of DMT at different doses and times after reaching peak DMT levels in brain are compared. The DMT concentration in rat liver is plotted on the x-axis and the DMT concentration in rat brain on the y-axis. Note that a correlation coefficient of 0.97 was obtained. Each point represents a single experiment.

DISCUSSION

The slight modification of the extraction procedure of Cohen and Vogel [25] enabled us to analyze DMT in tissues for a longer period than that previously reported. The extraction procedure is standard for the recovery of *N,N*-di-, -mono- or -unsubstituted-3-indolealkylamines such as T, NMT and DMT. Therefore, this assay is not specific for DMT alone, which is in agreement with Gross and Franzen [29] but disagrees with Cohen and Vogel [25]. Within the sensitivity limits of this assay procedure, there was negligible interference from endogenous indolealkylamines. *N*-demethylation of DMT has been proposed for its biological disposition [30]. To check whether this assay procedure could be utilized for the assay of injected DMT, tissue extracts were treated with trifluoroacetic anhydride, which converted primary and secondary amines to their corresponding amides. They could thus be separated from DMT by extracting DMT into an acid. The recoveries of tissue extracts treated with or without TFAA were not statistically significant from each other. Therefore no attempt was made to separate T and NMT. Hence this assay procedure was adapted for the purposes of this study.

A significant difference in the half-life of DMT in rat brain was noted after administration of two different doses. This suggests that different modes of clearance of DMT exist in the brain. Larger doses (10 mg/kg) of DMT resulted in a slower rate of clearance. This probably resulted from monoamine oxidase inhibition by the high doses of DMT, for it is known that tertiary amines can be weak MAO inhibitors and that DMT inhibits this enzyme [31].* There was no significant difference in the half-life of DMT in the liver after 3.2 or 10 mg/kg ($P > 0.05$). Although liver MAO might also be inhibited by DMT *in vivo*, this is not the only enzyme in the liver which can biotransform DMT. On the other hand, it is generally assumed that amines in brain are biotransformed chiefly by MAO. The half-life of DMT was greatly increased in both the brain and liver after pretreatment with iproniazid and tranylcypromine. This might be expected, for DMT is known to be converted to indoleacetic acid by mitochondrial and microsomal enzymes [32, 33]. This suggests that DMT is a substrate for MAO. It is of considerable interest that after MAO inhibition by either of these agents, the half-life of DMT in brain and liver is not very different ($P > 0.05$). This is strong evidence of similar modes of clearance of DMT from both the brain and liver after MAO inhibition. Perhaps after MAO inhibition, brain DMT is chiefly biotransformed by enzymes other than MAO. Tranylcypromine prolonged DMT half-life more than iproniazid under the present experimental conditions ($P < 0.05$).

The fact that the time during which DMT could be detected in the brain correlates with the duration of cessation of barpressing behavior suggests that the behavioral deficit is due to the direct involvement of DMT itself rather than a secondary effect of the drug, such as the formation of a metabolite [34] or its interaction with 5-HT [23]. The data are consistent with a direct central action of DMT.

* E. F. Domino and R. R. Krause, unpublished observations.

Acknowledgements The authors would especially like to thank Dr. Beverly Kovacic for permission to quote her behavioral data in this study. We extend our thanks to Dr. Sumathy Sampath-Khanna for her helpful suggestions and consultations. We gratefully acknowledge the efforts of Dr. Robert Moore, Ms. Ann Wilson, Ms. Sandra Demetriou and Ms. Diane Rufling. We are also indebted to Ms. Grace Seegert for typing this manuscript, Mr. Michael Lutz for preparing the illustrations and Mr. George Hovey of the Lafayette Clinic Computing Laboratory for the data analysis.

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