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A Comparison of the Behavioral Effects of Proteo- and Deutero-N, N-Dimethyltryptamine

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BEATON, J. M., S. A. BARKER AND W. F. LIU. *A comparison of the behavioral effects of proteo- and deutero-N,N-dimethyltryptamine*. PHARMAC. BIOCHEM. BEHAV. **16**(5) 811–814, 1982.—The behavioral effects of N,N-dimethyltryptamine (DMT) and α, α, β, β-tetradeutero-N,N-dimethyltryptamine (D_4DMT) at dose levels of 2.5 and 5.0 mg/kg were examined in rats on a food reinforced schedule. The D_4DMT was observed to produce a significantly greater disruption of behavior, have a longer duration of action and a shorter time to onset than DMT. This potentiation, apparently due to the kinetic isotope effect, suggests that DMT is significantly metabolized and deactivated by deamination at the α-position.

Proteo-N,N-dimethyltryptamine Tetradeutero-N,N-dimethyltryptamine Kinetic isotope effect
Behavior disruption

IN the last decade the hallucinogen N,N-dimethyltryptamine (DMT) has been identified as a normal constituent of human blood [1, 5, 12, 16, 19, 28], urine [7, 20, 21, 22], and cerebrospinal fluid (CSF) [8, 11, 26] and has subsequently been characterized as a putative neurotransmitter or neuromodulatory substance in rat brain [9]. A necessary process in the study of this endogenous hallucinogen has been the further elucidation of the mechanisms involved in its biosynthesis and metabolism. The indole-N-methyltransferase enzymes capable of synthesizing DMT from tryptamine and S-adenosylmethionine have been described and characterized in human lung, brain, blood and CSF and in various other mammalian species [2]. However, some questions have remained regarding its metabolism.

It has been reported that the pathways for DMT metabolism *in vivo* are 6-hydroxylation, occurring exclusively in the periphery, N-demethylation and deamination by monoamine oxidase (MAO) [2]. The most important pathway for DMT metabolism has been stated to be the action of MAO and aldehyde dehydrogenase on DMT to give indoleacetic acid (IAA), which has been measured as the major *in vivo* and *in vitro* metabolite of DMT [2]. The predominance of this pathway in DMT metabolism has been illustrated in several studies which reported a potentiation of the behavior disrupting properties and the tissue levels of DMT following inhibition of MAO [14, 17, 18, 23]. However, other studies have shown that DMT is not appreciably metabolized by purified preparations of MAO and that DMT is itself a MAO inhibitor [4, 13, 24]. Furthermore, Barker *et al.* [3] have reported recently that while pargyline inhibits the *in vitro* formation of IAA by 83% it also inhibits the demethylation and N-oxidation of DMT by 90%. Thus, the reported potentiation of the behavior disrupting effects and tissue levels of DMT by pargyline pretreatment may not have been due solely to inhibition of MAO. This matter is further complicated by the fact that IAA may also rise from the major intermediary metabolites of DMT, namely N-methyltryptamine (NMT) and DMT-N-oxide (DMT-NO) [3], and thus may not be totally derived from the deamination of DMT by MAO directly.

Since the mechanisms for the deamination of indolethylamines by MAO are proposed to involve the breaking of the α carbon-hydrogen (C-H) bond, the use of deuterium (D) in this position would be expected to slow the rate of deamination. This effect is due to the greater bond energy of C-D versus C-H bonds and is known as the kinetic isotope effect [6]. The use of deuterium thus allows one to examine the relative importance of certain parts of a molecule in its metabolism and thus to evaluate metabolic pathways and mechanisms. In this regard, the mechanisms for the other pathways (N-demethylation, N-oxidation and 6-hydroxylation) [3] in DMT metabolism are not proposed to involve the α C-H bond [2,3]. Therefore, α, α, β, β-tetradeutero-DMT (D_4DMT) might be expected to produce

a potentiation of the behavior disrupting properties in comparison to DMT, via the kinetic isotope effect *in vivo*. Such a potentiation would provide an indication of the involvement of deamination of DMT directly in its overall metabolism and deactivation. We report here a comparison of the behavior disrupting effects of DMT and D_4DMT using a food reinforced schedule. In this paradigm DMT disrupts behavior in an all-or-none response pattern which is dose dependent [10,15]. The duration of this disruption to initiate responding is an increasing function of dose and is, perhaps, a good measurement of the duration of action of DMT.

METHOD

Materials

The DMT and D_4DMT used in this study were provided by Professor Fred Benington and Dr. Richard Morin of this laboratory. The D_4DMT was obtained by the method of Speeter and Anthony [27] using lithium aluminum deuteride. Gas chromatographic/mass spectrometric analysis of the heptafluorobutyryl derivative of D_4DMT [3] gave a single peak with a calculated isotopic purity of 99.7%. All other materials were obtained from commercial sources.

Procedure

Sixteen adult, male, Long-Evans hooded rats served as subjects. The animals were housed singly in a room with a 12 hour light/dark cycle, and the temperature was maintained at 72°F±2°. The subjects weighed between 280 g–350 g on arrival from the supplier (Charles River). After a period of 10 days habituation to the laboratory with ad lib food and water, the animals were weighed on the next three days. The average of these three weighings was taken as the 100% weight of the animals. Over the next 14 days the weights of the animals were reduced to 80–85% of the free-feeding weights, by limited daily feeding. During this period water was available in the home cage ad lib.

The study was carried out using two Standard Lehigh Valley one-lever rat Skinner boxes. The chambers were placed in sound attenuated chests in a sound-proofed room. The programming equipment, consisting of solid state circuitry, cumulative recorders and tape programmers, was situated outside this room.

After initial training to bar press for food reinforcement, the animals were placed on a variable schedule of 30 sec (VI 30). The sessions were of 60 min duration. The subjects were trained until they reached the following criteria of stability:

(a) three consecutive sessions with less than 15% variation in the total number of responses.

(b) three consecutive sessions in which the number of reinforcements obtained was 112±5. (Although the theoretical maximum number of reinforcements in a 60 min session is 120, most subjects obtain around 115 reinforcements.)

When the animals had reached these criteria drug testing was begun. D_4DMT and DMT were tested at 2.5 and 5.0 mg/kg in two groups of eight rats. One level of drug was tested in any rat once per week, with a saline session preceding and following the drug test. All injections were in a volume of 0.1 ml/100 g body weight. The D_4DMT and the DMT were dissolved in 0.1% HCl in saline, with the pH being adjusted with 0.1 n NaOH. The final solution had a pH of 6 to 7.

The total number of responses and reinforcements were noted per session and a cumulative record was obtained of the response and reinforcement distributions.

RESULTS

Table 1 (a) shows the mean number of responses made and reinforcements obtained, plus or minus the standard deviation, for all test conditions. As can be seen from these data there is a high standard deviation for the number of bar presses, reflecting the individual differences in bar pressing rate. However, the number of reinforcements obtained has a much lower standard deviation, and since on a VI schedule this measure is relatively independent of bar pressing rate, *t*-tests were carried out on these data. The paired *t*-test was used for comparisons of saline and drug within the same subject, and the *t*-test for independent means was used for comparisons between drug effects. It can be seen that all levels of DMT and D_4DMT were significantly different from saline (Table 1(a)). Also, the comparisons between DMT and D_4DMT at the same dose level are significantly different (Table 1(b)).

The time of onset of drug disruption and the duration of drug disruption were calculated from the cumulative records. Latency of drug disruption was defined as the period between injection of drug, immediately before the start of the experimental session and the point in time at which lever-pressing was completely abolished, for 1 min or more. Resumption of VI responding was defined as the point at which bar-pressing was resumed abruptly. Duration of disruption was defined as the period between the cessation and the resumption of a steady rate of lever-pressing.

Table 2 (a) shows the mean latency for DMT and D_4DMT to induce disruption and the mean duration of such disruption. For all conditions the onset of DMT's disrupting effects was very rapid, within 2–3 minutes. *t*-Tests for independent means were carried out on the comparisons of DMT and D_4DMT. The results of these can be seen in Table 2(b). All comparisons of DMT effects with D_4DMT effects were significantly different for both latency of onset and duration of action.

DISCUSSION

The potentiation of the behavior disrupting effects of DMT by substitution of D for H on the α and β carbons of the ethylamine side chain is very similar to that observed by pretreatment of test animals with a MAO inhibitor [14, 17, 18, 23]. This potentiation and the prolongation of disruption may be directly attributed to the kinetic isotope effect, possibly leading to higher levels and a longer half-life ($t^1/_2$) for D_4DMT in brain [6]. Similarly, the decreased latency to onset of disruption may be due to the ability of D_4DMT to attain threshold levels for disruption more rapidly than DMT itself. The paradigm selected for use in this study was chosen because of the easy quantification of the "all-or-none" disruptive pattern [10,15], but the effects should be cross-validated using another behavioral procedure, e.g., the discriminated Sidman avoidance schedule which has been used frequently in the study of hallucinogens [25].

From the present study, it would appear that DMT is significantly metabolized and deactivated by deamination directly at the α position. The possible importance of this pathway is underscored by the observation that inhibition of microsomal enzymes by SKF-525-A does not lead to a significant potentiation of the tissue levels or pharmacological effects of DMT [23]. Although the pathway for the

TABLE 1

THE MEANS, PLUS OR MINUS THE STANDARD DEVIATIONS, FOR THE NUMBER OF BAR PRESSES AND REINFORCEMENTS FOR ALL PRE-DRUG SALINE AND DRUG CONDITIONS

(a)

Drug Dose (mg/kg)	No. Responses (Mean±S.D.) Saline	No. Responses (Mean±S.D.) Drug	No. Reinforcements (Mean±S.D.) Saline	No. Reinforcements (Mean±S.D.) Drug	*p* Value
DMT 2.5	2,062±293	1,730±305	113±2	105± 4	<0.05
D_4DMT 2.5	1,651±240	805±192	115±1	66±10	<0.001
DMT 5.0	1,941±290	1,173±160	114±1	86± 6	<0.01
D_4DMT 5.0	1,870±388	648±160	115±1	52± 5	<0.001

(b)

t-TEST COMPARISONS OF DMT AND D_4DMT

Comparison	*p* Value
2.5 mg/kg DMT vs D_4DMT	<0.01
5.0 mg/kg DMT vs D_4DMT	<0.001

TABLE 2

THE MEAN PLUS OR MINUS THE STANDARD DEVIATION FOR THE LATENCY TO, AND DURATION OF, THE ALL-OR-NONE DISRUPTION SEEN WITH DMT AND D_4DMT

(a)

Drug and Dose	Latency	Duration
DMT 2.5 mg/kg	3.4±0.7	12.5±1.6
D_4DMT 2.5 mg/kg	1.3±0.4	21.7±1.6
DMT 5.0 mg/kg	1.3±0.5	23.8±2.7
D_4DMT 5.0 mg/kg	0.6±0.2	31.3±3.5

(b)

t-TEST COMPARISONS OF DMT AND D_4DMT

	Comparison	*p* Value
Latency	2.5 mg/kg DMT vs D_4DMT	<0.01
	5.0 mg/kg DMT vs D_4DMT	<0.05
Duration	2.5 mg/kg DMT vs D_4DMT	<0.001
	5.0 mg/kg DMT vs D_4DMT	<0.01

N-oxidation of DMT may not be significantly affected by this drug [2], this finding may be taken as an indication that the formation of the other metabolites of DMT play an apparently minor role in its deactivation and that the major enzyme involved in DMT metabolism is indeed MAO.

The use of a deuterated analog may also effect the metabolism, uptake and/or binding of DMT in the periphery as well as the central nervous system. Thus, other physiological processes may be altered by the kinetic isotope effect and these too may play a part in affecting the pharmacokinetic profile of DMT. However, preliminary gas chromatographic/mass spectrometric analysis of rat brain levels of injected DMT or D_4DMT indicate that D_4DMT is more rapidly taken up into brain tissues, attains a level 2–3 times greater than that observed for DMT and has a longer $t^1{}_2$ than DMT itself (Barker *et al.*, in preparation). These preliminary findings are in agreement with the behavioral results reported here and the possibility that D_4DMT metabolism, in the periphery and the central nervous system, may be significantly different from that of DMT itself. Although the effect of deuteration on DMT binding is not known at present, one might expect that the D_4DMT would also exhibit a different affinity for binding. Since this binding affinity could be either higher or lower, we cannot at present address the possible effects of binding differences on the observed behavioral effects.

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