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The *in Vitro* Identification of Dimethyltryptamine (DMT) in Mammalian Brain and Its Characterization as a Possible Endogenous Neuroregulatory Agent*

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For some time there has been a controversy as to whether or not the psychotogen dimethyltryptamine (DMT) is an endogenous constituent of mammalian brain (1). The answer to this question is particularly relevant when viewed in the light of theoretical considerations which postulate that DMT is the etiological agent responsible for schizophrenia (2). Dimethyltryptamine is an excellent candidate for such a "schizotoxin" since, unlike many of the other hallucinogenic agents, tolerance to DMT does not seem to develop in any predictable manner. In fact, studies in both cats (4) and squirrel monkeys (5) have shown that no measurable tolerance develops to DMT even when high doses are given. Studies in man are somewhat more complex, with subjects exhibiting a variable or aperiodic partial tolerance to DMT (5). Although a vast amount of literature has developed around the idea of DMT as an endogenous psychotogen (2, 5), few convincing data have appeared which actually identify DMT as an endogenous compound in the brain of either man or animals. Consequently, the purpose of this study centers around the *in vivo* identification of DMT and its direct precursor tryptamine in the brains of rodents. In addition to the identification of DMT as an endogenous compound in brain, we have also determined the subcellular localization of the compound. From determinations of the ability of DMT to stimulate adenylate cyclase in synaptosomal membranes as well as other considerations, we have postulated the idea that DMT may be a naturally occurring neuroregulatory agent or perhaps even a neurotransmitter *per se*.

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MATERIALS AND METHODS

Materials

d-[^{3}H]Lysergic acid diethylamide was obtained from the New England Nuclear Corporation (17.1 Ci/mmole) and from Amersham/Searle (21.0 Ci/mmole). Tritiated cyclic AMP (27.5 Ci/mmole) was also obtained from the latter company. Toluene was obtained from the J. T. Baker Chemical Company and from Burdick and Jackson Laboratories. Methylene chloride was also obtained from Burdick and Jackson. Triton X-100, 2,5-diphenyloxazole (PPO), 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene (dimethyl POPOP), tryptamine hydrochloride, serotonin binoxylate (5-HT), sucrose, adenosine 5′-triphosphate (ATP), theophylline, ethyleneglycol-bis(B-aminoethylether)-*N*,*N*-tetracetic acid (EGTA), and dopamine hydrochloride were obtained from the Sigma Chemical Company. Pargyline was obtained from Abbott Laboratories and Ficoll was obtained from Pharmacia Fine Chemical Incorporated. Cellulose acetate filters (0.45 μm pore size) with a diameter of 25 mm were obtained from the Nucleopore Corporation. Cyclic AMP specific binding protein was obtained from Calbiochem. Dimenthyltryptamine, *O*-methylbufotenine and *d*-LSD were obtained from the National Institute of Mental Health. Heptafluorobutyryl imidazole was obtained from Pierce Chemical Company and the pure heptafluorobutyryl derivatives of dimethyltrypatmine and tryptamine were prepared by us as previously described (6). All other reagents and materials were of the highest available purity.

Subcellular Fractionation

Synaptosomal membranes were prepared from whole rat brains using a modification of the method described by Cotman and Matthews (7). Male Sprague–Dawley rats weighing between 200 and 300 g were used as the tissue source. The animals were fed and watered *ad libitum*.

Animals were sacrificed by decapitation and their brains were removed as quickly as possible and immediately homogenized at 200 rpm in 0.32 M sucrose, pH 7.4, containing 100 μM $CaCl_2$, using a glass homogenizer with a motor-driven Teflon pestle (0.25 mm clearance). All operations were carried out at 4°. The scheme used for the preparation of lysed synaptosomal membranes from brain homogenates is shown in Fig. 1. Whole cells, nuclei, and cell debris were sedimented by centrifugation at 1000 *g* for 10 min. The supernatant (S_2) was then centrifuged at 10,000g for 20 min in a Sorvall RC-2B preparative centrifuge. The pellet from this step was resuspended by homogenization in a small volume of the original homogenizing medium and layered onto a discontinuous Ficoll–sucrose gradient composed of 6 and 13% Ficoll in 0.32 M sucrose containing 100 μM calcium chloride. The gradient solutions were then centrifuged in a

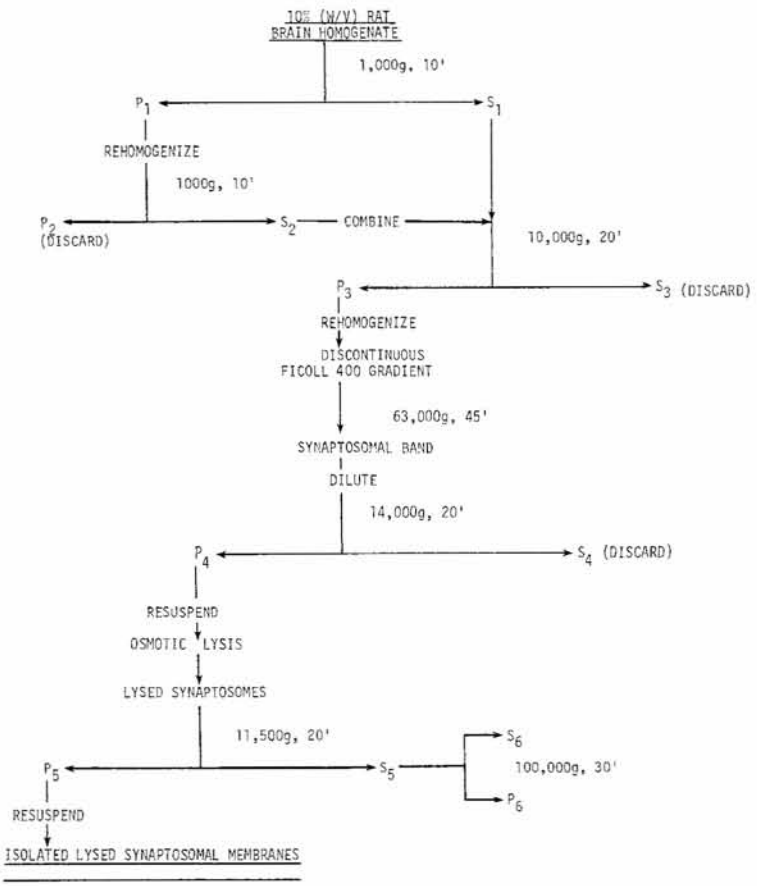

FIG. 1. Schematic diagram of the fractionation of various subcellular components from whole rat brain homogenates.

SW-27 swinging bucket rotor in a Beckman L-50 ultracentrifuge at 63,000*g* for 45 min. The band which sedimented at the 6/13% (Ficoll–sucrose) interface was removed by aspiration, resuspended in 0.32 M sucrose containing 100 μM $CaCl_2$, and pelleted at 14,000*g* for 20 min. The pellet (P_4) from this step was then resuspended in a small volume of 0.32 M sucrose and lysed by gentle homogenization in a 50-fold excess (v/v) of 0.005 M tris(hydroxymethyl) aminomethane (Tris), pH 8.0, buffer containing 10μM $CaCl_2$ for 1 hr. Following centrifugation at 11,500*g* for 20 min the lysed synaptosomal membrane fraction (P_5) was resuspended in isotonic saline, which was 0.005 M in Tris buffer, pH 7.4, 5×10^{-4} M in pargyline and 100 μM in calcium chloride. The supernatant (S_5) from the 11,500*g* spin contains primarily synaptic vesi-

cles. Vesicles were isolated from the S_5 supernatant by centrifugation at 100,000*g* for 30 min using a Beckman Type-40 rotor. This centrifugation step concentrates the vesicles into the pellet which we designated P_6. The supernatant (S_6) from this step was utilized in later experiments. All protein determinations were carried out by the method described by Lowry *et al.* (8). Various marker enzymes were determined as follows: lactic dehydrogenase (9), cytochrome *c* oxidase (10), Na^+, K^+-activated ATPase, and Mg^{2+} activated ATPase (11, 12). Results from the marker enzyme determinations gave the results one would expect from this type of cell fractionation with the highest Na^+,K^+-ATPase activity in the lysed synaptosomal fraction and the highest Mg^{2+}-ATPase activity in the vesicular fractions (13).

Adenylate Cyclase Activity

The method utilized in this study is a modification of the assay system reported by Kebabian *et al.* (14). The assay medium contained, in millimoles per liter: Tris buffer (pH 7.4), 80; adenosine 5′-triphosphate, 0.5; $MgSO_4$, 2.0; theophylline, 10; EGTA, 0.5; synaptosomal membranes (0.1 to 0.5 mg); and the compound under investigation. The total volume of the incubation medium was 0.5 ml. All samples were run in duplicate. The reaction was initiated by the addition of ATP and was allowed to proceed for 20 min in a 30° water bath. The amount of adenosine 3′,5′-monophosphate (cAMP) produced under these conditions was found to be linear for at least 20 min if the protein concentration did not exceed 0.5 mg/ml. The reaction was terminated by placing the reaction tubes along with the proper controls (e.g., controls for the nonenzymatic production of cAMP) in a boiling water bath for 10 min. The amount of cAMP produced was determined by a modification of the method described by Gilman *et al.* (15). The amount of cAMP formed during the incubation period was determined on duplicate 0.05-ml aliquots of this solution. The assay method depends on a highly specific binding reaction between cAMP and a protein isolated from rat liver. Binding protein used in our assays was obtained as a lyophilized powder from Calbiochem. The resulting cAMP–protein complex is capable of binding to a cellulose nitrate filter disk. One hundred microliters of a 0.02 M phosphate buffer solution, pH 6.0, containing [^{3}H]-cAMP (111,000 dpm or approximately 50,000 cpm) was added to each 0.05-ml aliquot of the incubation medium. A convenient vessel for the cAMP assay is a 10 × 75-mm disposable culture tube. After the addition of radiolabeled cAMP, 50 μl of the binding protein (0.02 mg) in 0.02 M, pH 6.0, phosphate buffer is added to each assay tube. The addition of binding protein initiates the displacement reaction sequence. The reaction tubes are then incubated for 75 min in an ice bath. Mass action causes the displacement of the bound [^{3}H]-cAMP from the

binding protein. Consequently, the more cAMP that was made in the incubation reaction with ATP, the more [^{3}H]-cAMP will be displaced. After the 75-min equilibration period, each reaction tube is brought to a volume of 1 ml by the addition of 0.8 ml of 0.02 M phosphate buffer, pH 6.0. The solution is then filtered by suction through a 0.45-nm cellulose nitrate filter. The filter, which now has the binding protein adhering to it, is dissolved in 5 ml of a liquid scintillation cocktail consisting of 16.5 g of PPO, 0.3 g of dimethyl POPOP, 1 liter of Triton X-100 in 2 liters of toluene. The amount of radioactivity still bound to the binding protein is inversely related to the amount of cAMP produced. The number of disintegrations per minute in each sample is compared to a standard curve constructed using known amounts of cAMP. The results may then be expressed as the number of picomoles of cAMP formed in each sample. Radioactivity was measured using a Beckman LS-250 liquid scintillation spectrometer.

Equilibrium Dialysis Studies

The binding of various ligands to sites on lysed synaptosomal membranes was measured using the technique of equilibrium dialysis (16, 17). For these studies the Kontron Diapack equilibrium dialysis system (Kontron) was utilized. This apparatus consists of all Teflon dialysis cells having half-cell volumes of approximately 1 ml. In this system five Teflon cells are mounted in a rotating stack. Four of these stacks represent a total of 20 dialysis cells. The four stacks are held in a special motor driven unit which rotates each stack at 4–5 rpm. Each dialysis cell is separated by a cellulose membrane which had been washed in boiling 0.1 M $NaCO_3$, 0.1 M EDTA, glass-distilled H_2O, and finally in the dialysis buffer. The dialysis buffer consists of 0.005 M Tris buffer, pH 7.4, 0.15 M NaCl, 100 μM $CaCl_2$, and 5×10^{-4} M pargyline. Pargyline is added as a precaution in order to inhibit any monoamine oxidase present in the system from mitochondrial membrane contamination. Lysed synaptosomal membranes were added to one side of the cells at a concentration ranging from 0.1 to 0.5 mg/ml of membrane protein. Binding was found to be linear within this range. Radioactive ligands or antagonist were also added to the side of the dialysis cell which contained the synaptosomal membranes. The advantage of using the all Teflon apparatus is reflected in the absence of nonspecific binding to the dialysis cell itself. Controls also showed that virtually no binding occurred between the ligand and the cellulose membrane. Consequently, it was not necessary in these studies to correct for nonspecific binding. After the cells were loaded with membranes and the ^{3}H-labeled ligand to be studied, the system was allowed to equilibrate for 18 hr at 4°. After the equilibration period, the amount of bound ligand is determined by counting the amount of radioactivity on each side of the

dialysis membrane. The liquid scintillation cocktail used has already been described.

Determination of DMT and Tryptamine

As previously described, vesicles (P_6) are sedimented from the S_5 supernatant by centrifugation at 100,000*g* for 30 min. The supernatant S_6 is then lyophylized to dryness. Six rat brains (approximately 12 g of tissue) yield a sufficient amount of vesicular protein, which usually contains an easily quantifiable concentration of DMT and tryptamine using the gas chromatographic technique. The glc method described here is an adaptation of a technique recently reported by us (18). Both the vesicle pellet and the lyophylized supernatant are each suspended in 2 ml of glass-distilled water. The protein is then precipitated by 7% perchloric acid and after centrifugation sufficient 2 N NaOH is added to the protein-free solution to bring the pH to 12.0. A saturating amount of NaCl is then added to the solutions and they are extracted with 2 × 3 ml of ultrapure methylene chloride. The methylene chloride extract is dried with Na_2SO_4. The methylene chloride, containing DMT and tryptamine, is taken to *dryness* in a 5-ml conical reaction flask on a rotary evaporator. This step is somewhat critical since the residue must be concentrated in the bottom tip of the flask. After evaporation 20 μl of heptafluorobutyryl imidazole is added to the flask. A glass stopper is immediately inserted and the mixture is allowed to react neat for 1 hr at 85° (Wood's metal bath). After cooling, 0.5 ml of glass-distilled H_2O is added to decompose any excess reagent. The unreacted reagent decomposes into heptafluorobutyric acid and imidazole. The 0.5 ml of H_2O added is sufficient to dissolve any imidazole formed. Heptafluorobutyric acid is removed in a subsequent step. Two milliliters of ultrapure redistilled toluene is then added to the reaction flask. The two-phase system is then transferred to a 15-ml conical centrifuge tube. The bottom aqueous layer is carefully removed with a Pasteur pipet and discarded. The toluene solution containing derivatized DMT, tryptamine, and any other indolealkylamines which may be present is washed three times with 0.5 ml of glass-distilled H_2O. After the final wash the toluene solution is centrifuged in a clinical desk-top centrifuge to separate the last traces of entrained water. Three microliters of this solution are then injected into a Hewlett–Packard Model 5700A gas chromatograph equipped with a ^{63}Ni electron capture detector. The chromatographic conditions will be given in the legend to Fig. 2. The emergence time of the unknown sample is directly compared to that of pure crystalline heptafluorobutyryl (HFB) dimethyltryptamine and tryptamine standards. The preparation of these standards as well as other indolealkylamine standards is reported in a previous publication (6). As an additional check a "spike" of the pure derivatized standard equal to the

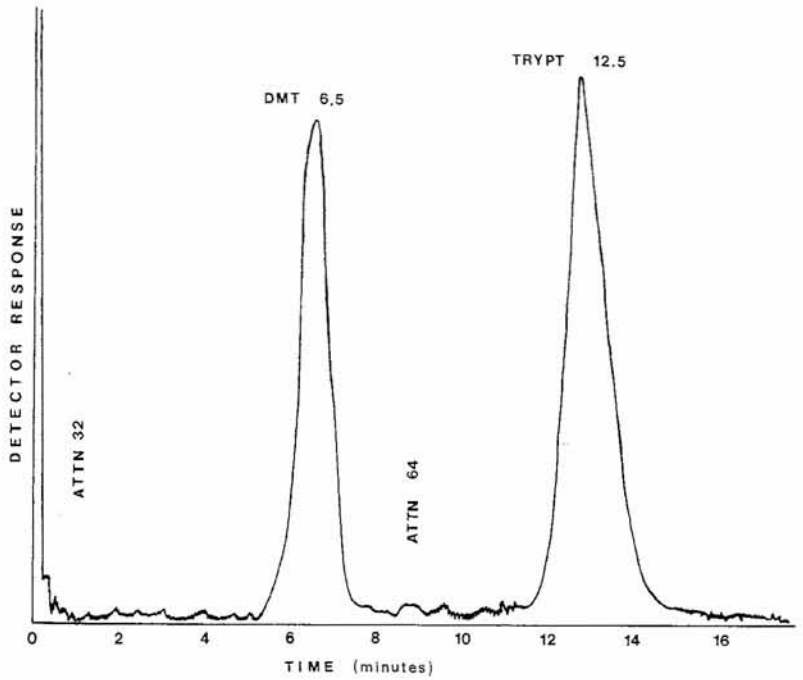

FIG. 2. Gas chromatogram of the heptafluorobutyryl derivatives of tryptamine and dimethyltryptamine. The pure compounds were derivatized, isolated, and purified according to (6). The glc column used was a 1.8 × 2 mm i.d. glass column packed with 3% OV-17 on 80-100 mesh Gas Chrom. Q. Three microliters of a toluene solution containing 200 pg of each standard was injected directly onto the column. Instrument conditions were as follows: injection block temperature, 250°; oven temperature, 175°; gas flow (95% argon/5% methane), 50 cm^3/min; detector temperature, 300°. The retention times as shown in the figure are 6.5 min for DMT and 12.5 min for tryptamine.

quantity of DMT and/or tryptamine found in the respective samples is injected. If no shoulder appears at the correct retention time and if the peak area doubles, the unknown material is said to be identical to the standards. The amount of DMT present in the sample is determined by comparing the peak area of the unknown with the peak area of a known amount of the standard. Detector response was found to be linear from at least picograms to nanograms of either HFB–dimethyltryptamine or HFB–tryptamine. We should like to stress the use of ultrapure solvents in the gas chromatographic procedure. Glass-distilled solvents were obtained from Burdick and Jackson Laboratories and were further purified by redistillation on a 40-plate Todd column at a reflux ratio of 10:1. Only the "heart cut" from this redistillation is utilized for the glc procedure. It should be mentioned here that our glc technique for the detection of DMT

and other indolealkylamines has been verified (on a qualitative basis) by the LKB Instrument Company on their LKB 2091 mass spectrometer (19). In addition to this verification another laboratory has independently verified the presence of DMT in DMT positive samples of human cerebrospinal fluid using the method of isotope-dilution mass fragmentography (20).

RESULTS

Identification of DMT and Tryptamine in Isolated Vesicles

Figure 2 is a gas chromatogram showing the emergence times and relative peak heights of a toluene solution containing 200 pg of pure HFB–dimethyltryptamine and 200 pg of HFB–tryptamine. The emergence time for DMT is 6.5 and 12.5 min for tryptamine. Figure 3 is the chromatogram derived from the methylene chloride extract of the vesicular supernatant (the S_5 fraction) obtained from six rat brains. This

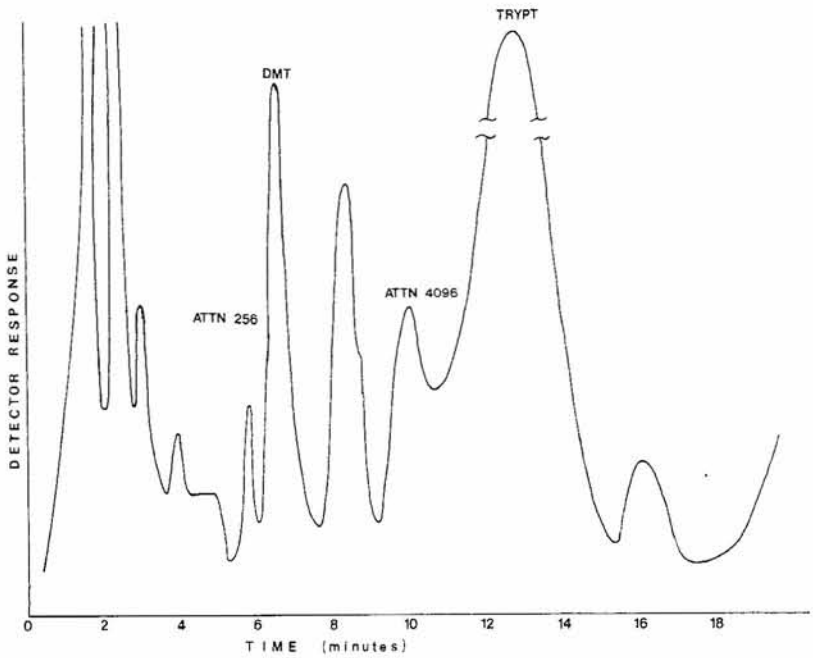

FIG. 3. The gas chromatogram of the methylene chloride extracted S_5 vesicular fraction. Changes in instrument sensitivity are noted in the figure as changes in attenuation. The peaks at 6.5 and 12.5 min indicate 5.8 ng of DMT/mg of protein and 40 ng of tryptamine/mg of protein in the injected sample. The *y*-axis is expressed as the relative detector response. Extraction efficiencies for these two compounds were found to be better than 90+% for both compounds when known amounts of each compound were added to the tissue source (18).

chromatogram indicates the presence of DMT at an apparent concentration of 4.6 ng/mg of vesicular protein and tryptamine at a concentration of 40 ng/mg of protein. If the toluene solution is now spiked with the pure HFB standards for each compound, no change is seen in retention time for either compound and the increase in peak area is equal to that which would be expected from standard calibration curves. As a further check on quantitation, however, since the sensitivity of the detector may vary slightly from day to day, standards are run each day and the quantity of each compound is calculated as a ratio of standard area versus the area of the compound of interest. Results from three separate experiments are shown in Table 1. As already indicated under Materials and Methods the vesicle-rich S_5 supernatant is further subdivided into the vesicle pellet (P_6) and the vesicle-depleted supernatant as the (S_6) fraction. The results of both Experiments 1 and 2 (in Table 1) indicate that the amount of DMT is approximately equal in both vesicles (P_6) and the vesicle supernatant (S_6) while tryptamine was found to be primarily located in the supernatant from the 100,000*g* spin with a relatively small amount in the vesicle pellet. In Experiment 3 (Table 1) if Mg^{2+} and ATP were added to the (S_5) vesicle containing supernatant and the mixture was allowed to incubate for 30 min at room temperature almost all of the DMT was then found in the (P_6) pellet with very little remaining in the (S_6) supernatant. The relative distribution of tryptamine, however, appeared to be unaffected by this treatment. That is, no transfer could be detected between supernatant and vesicles. This experiment would appear to demonstrate the active transport of DMT into brain vesicles in much the same way as transmitters are

TABLE 1
DISTRIBUTION OF DMT AND TRYPTAMINE IN THE VESICLE PELLET AND THE VESICLE SUPERNATANT

Experiment	Fraction	Protein concentration (mg/ml)	DMT (ng/mg)	Tryptamine (ng/mg)
1	S_6	3.0	5.4	40
	P_6	1.4	5.2	1.9
2	S_6	3.0	5.2	41
	P_6	0.8	5.4	1.1
3[a]	S_6	3.5	0.0	40
	P_6	1.7	11.2	0.7

[a] Experiment 3: In this experiment the S_5 supernatant containing the neuronal vesicles was incubated for 30 min at room temperature in the presence of 5×10^{-5} M ATP and 1×10^{-4} M $MgCl_2$. As described under Materials and Methods, before glc analysis the solution was centrifuged at 100,000*g* for 30 min to obtain the vesicle pellet (P_6) and the supernatant (S_6).

pumped into brain vesicles (21–23). The distribution of DMT in cellular fractions and its concentration in the vesicular material has been described in previous preliminary reports (24, 25). In order to preclude the possibility of binding rather than uptake the pellets were washed twice with isotonic buffered saline. No DMT could be detected in the washing solution. However, it should be clearly pointed out that the results of these experiments be considered as preliminary data. The possibility still exists at this point that DMT may be so tightly bound to the vesicular membrane that washing the particles with isotonic buffered saline will not dissociate the bound compound. Further, at this point, assuming that DMT is actively transported we have no idea as to the mechanism of transport.

Binding of Dimethyltryptamine to Synaptosomal Membranes

Since DMT was found in the rodent neuronal vesicles and since it has known central activity as a psychotomimetic agent (26), one might speculate that DMT would bind to synaptosomal membranes. In order to test this hypothesis, binding studies were carried out on lysed synaptosomal membranes. It is important to point out here that only the lysed membranes were utilized for the dialysis experiments since other investigators have shown that transmitter reuptake occurs in the intact synaptosome (23). Consequently, we did not wish to fall into the trap of confusing possible uptake of this agent with binding. Unfortunately we have not as yet been able to make labeled DMT with a high enough specific activity to directly measure its binding at high affinity sites on the membranes. In order to circumvent this problem we decided to use the high affinity *d*-LSD binding site to demonstrate competitive antagonism. *d*-LSD is readily available at high specific activities (17.1 Ci/mmole) and other investigators have suggested that owing to their structural similarities the two compounds may bind at the same synaptosomal site (27–29). Our equilibrium dialysis experiments with *d*[^{3}H]-LSD indicated that it binds to the isolated synaptosomal membranes with a K_d of 3.0×10^{-9} M. This value is ascertained from the Scatchard plot (30) shown in Fig. 4 and is very close to the value reported recently by Bennet and Aghajanian (31), i.e., 4×10^{-9} M. In our studies if 1×10^{-7} M *d*-LSD was allowed to bind to synaptosomal membranes at a membrane protein concentration of 0.5 mg/ml, the bound LSD could be completely displaced by 1×10^{-5} M DMT. We also examined other psychotogens of the indolealkylamine class to see if they would also displace bound LSD. If 5-methoxy-*N*,*N*-dimethyltryptamine (*O*-methylbufotenine) was added to the membrane suspension at a concentration of 1×10^{-5} M and the mixture was allowed to dialyze, complete displacement of the radioactive LSD was noted at the end of the dialysis run (see Materials and Methods section for details of the equilibrium dialysis procedure). These data suggested that DMT is

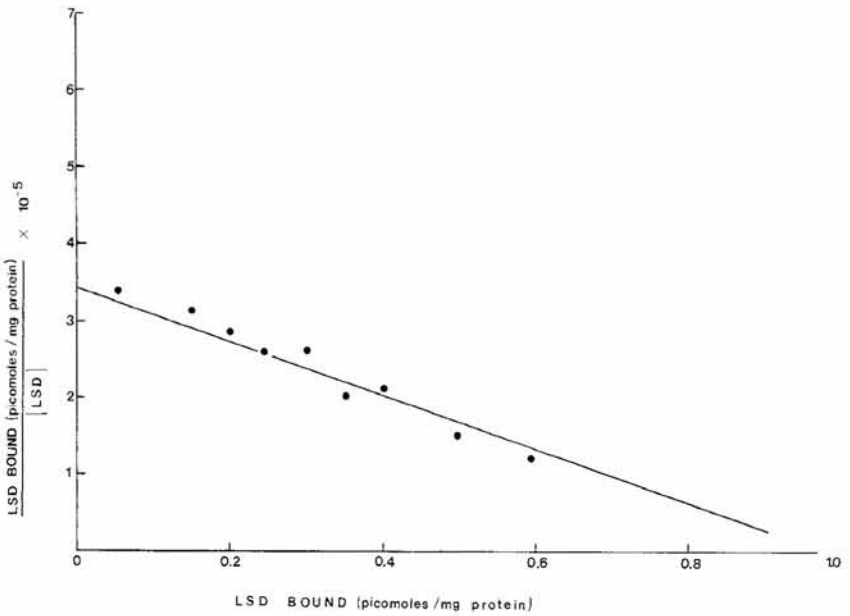

FIG. 4. Scatchard plot of the binding of *d*-lysergic acid diethylamide to lysed synaptosomes (P_5 fraction). Each point is the average of duplicate determinations. The line is established according to the method of least squares. From this plot a K_d of 3×10^{-9} M is obtained with the number of binding sites n approximately equal to 1 (0.98). Calculated values are per milligram of synaptosomal protein.

bound at the same site on the synaptosomal membrane as is LSD. The idea of a common psychotogen binding site is certainly not new. Smythies *et al.* (32) had proposed the idea of a common psychotogen binding site for DMT, LSD, and other compounds of this class as early as 1970. As a control, [^{3}H]-serotonin was likewise bound to lysed membranes and although more than one 5-HT binding site was observed the highest affinity site ($K_d = 2.4 \times 10^{-10}$ M) was taken as the test site in order to ascertain whether or not DMT would displace serotonin bound to this site. The serotonin K_d was ascertained from the Hill plot represented in Fig. 5. Results from the displacement experiment indicate that only a very small amount of [^{3}H]-serotonin ($\sim$ 10%) is displaced from this site with concentrations of DMT as high as 5×10^{-5} M. Since DMT at 1×10^{-5} M will completely displace LSD but not 5-HT, the data appear to suggest that (i) DMT binds to the LSD binding site and (ii) this site is different from the high affinity 5-HT site.

Stimulation of Synaptosomal Adenylate Cyclase Activity

The results obtained from the equilibrium dialysis studies suggest that if there are indeed different binding sites for 5-HT and DMT then one would

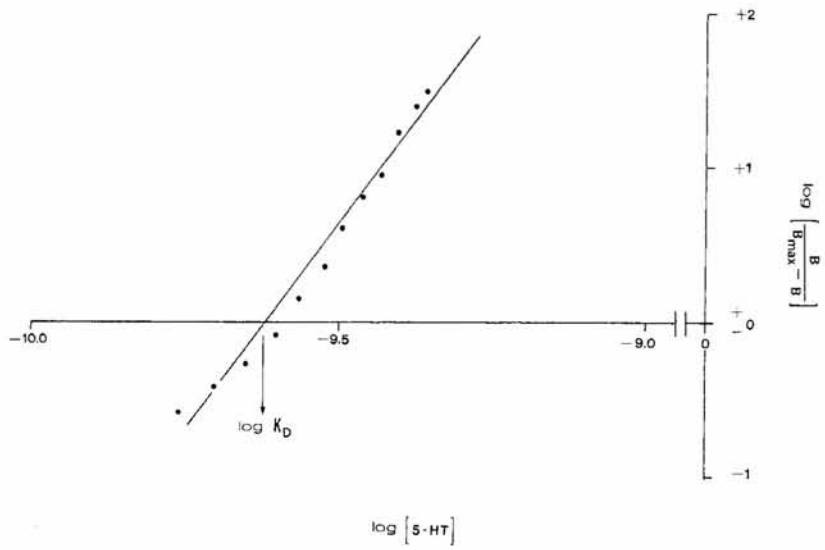

FIG. 5. Hill plot of the binding of serotonin to synaptosomal membranes. The x-axis is the common logarithm of the molar concentration of serotonin. Serotonin concentrations ranged from 1×10^{-9} to 1×10^{-10} M. The y-axis is the logarithm of the number of moles of 5-HT bound B/the maximum number of moles of 5-HT bound B_{max} minus B. Values of B are expressed as the number of moles of 5-HT bound per milligram of synaptosomal protein. The value of B_{max} was taken as 4.1×10^{-13} mole/mg of protein. From this plot a K_d for 5-HT is calculated at 2.4×10^{-10} M. The plot depicts a simple bimolecular binding reaction.

expect to see differences in the stimulation of adenylate cyclase when equimolar concentrations of each compound interacts with the membranes. De Robertis *et al.* (33) have already shown that this enzyme is concentrated in synaptosomal membranes. Figure 6 depicts the relative production of cAMP as a function of time when various ligands at a concentration of 5×10^{-10} M interact with the lysed synaptosomes. From Fig. 6 the relative rates of cAMP production in the presence of each ligand may be compared to the endogenous control rate. These data show that production of cyclic AMP decreases in the following order: dopamine > serotonin > DMT > LSD. However, the amount of cyclic AMP produced by DMT is not very different from that produced by LSD at equimolar concentrations. Tryptamine appears to be slightly inhibitory when compared to the endogenous control value. Other investigators have suggested that tryptamine has a neuroregulatory function (34, 35). These data do, however, clearly show that the endogenous concentration of DMT found in brain can stimulate adenylate cyclase activity presumably via the same mechanism as other known transmitters. In order to show further that DMT binds at a site distinct from the serotonin site various

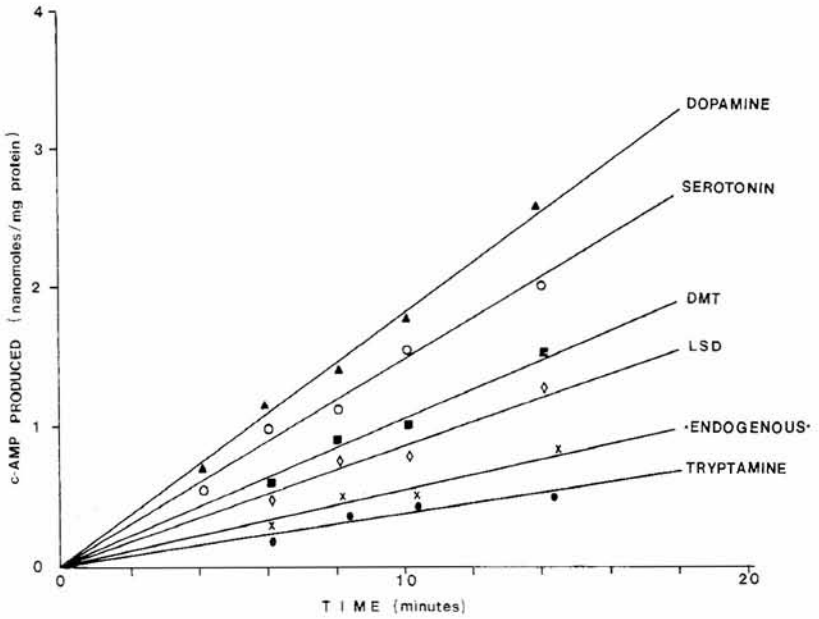

FIG. 6. The amount of cAMP produced in nanomoles per milligram of synaptosomal protein as a function of time in minutes. This plot indicates the relative production of cAMP by the various ligands at concentrations of 5×10^{-10} M, relative to the endogenous control. Details on the measurement of cAMP production are given under Materials and Methods.

combinations of equimolar concentrations of combinations of DMT, LSD, and 5-HT were examined as to their adenylate cyclase stimulatory activity. The results of this experiment are shown in Fig. 7. The combination of DMT with LSD produces an amount of cAMP (1.65 nmole/mg) in 20 min that is greater than with LSD alone but lower than the equimolar concentration (5×10^{-10} M) of DMT alone. These data would seem to indicate that LSD has a higher affinity for the DMT receptor site but a lower instrinsic activity. Therefore, the quantity of cAMP produced by these compounds in combination yields a concentration of cAMP intermediate between the two. If one were to assume that LSD and DMT bound to the 5-HT site then we would expect a combination of the two ligands to generate less cyclic AMP than is indicated in Fig. 7. In fact, the ligand combinations of 5-HT with either psychotogen appears to give rise to a slight synergistic effect. Even though we cannot explain this effect, these data do show that DMT and/or LSD is not blocking the 5-HT high affinity binding site at the 5×10^{-10} M concentration. However, in a separate study some blocking (approximately 30%) does occur if the concentration of LSD is 100 times higher than that used in this study (36). Along with these data as well as

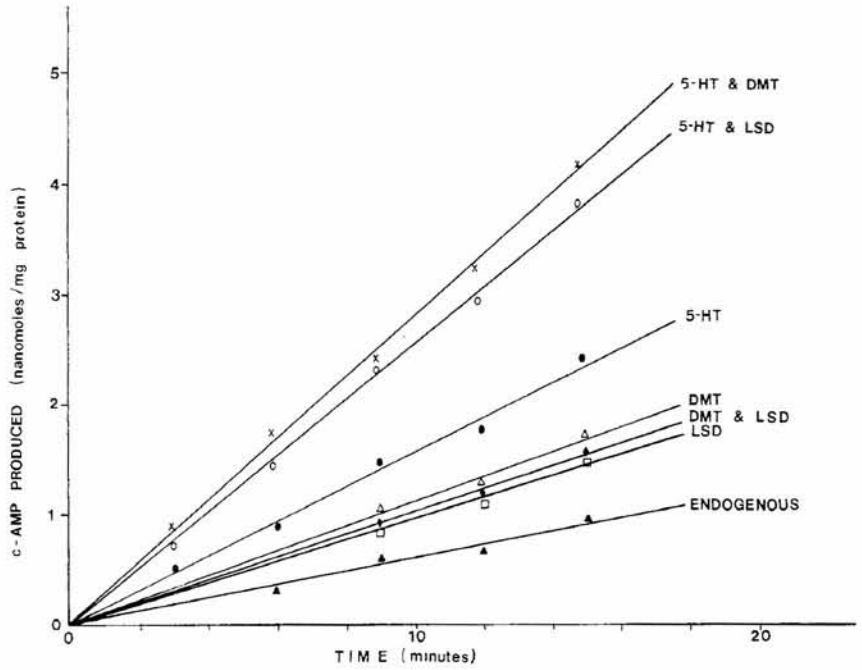

FIG. 7. The amount of cAMP produced as a function of various ligands at concentrations of 5×10^{-10} M. The axes are the same as those shown in Fig. 6. Each value is expressed as being relative to the endogenous control value. Notice that equimolar concentrations of DMT plus LSD yield an amount of cAMP lower than does DMT alone. This value would seem to suggest that LSD has a high affinity for the DMT binding site but a low intrinsic activity. However, equimolar concentrations of 5-HT plus LSD and 5-HT plus DMT yield values of cAMP production consistent with the hypothesis of separate binding sites on the synaptosomes for each compound.

the results of the previous experimentation it seems reasonable to assume that DMT has a specific binding site on the synaptosomal membrane and that as an endogenously occurring compound has a definitive *in vivo* function.

DISCUSSION

In practice, any chemical substance that is a normal constituent of nervous tissue and has a defined excitatory or inhibitory action on nerve or muscle cells is potentially classified as a neurotransmitter. Starting from this rather inclusive definition, we have presented data in this study on dimethyltryptamine, a common psychotomimetic agent, which demonstrate that the compound may be found in normal rodent brain and in a separate study in the cerebrospinal fluid of humans (19, 20, 37). The presence of what appears to be its direct precursor, tryptamine, has also

been found in both human and animal brain (38). One of us (39) using a gas–liquid chromatographic technique as well as the fluorometric method described by Hess and Udenfriend (40), had previously found nanogram quantities of this compound in human, steer, and dog brains. These finidings were later independently verified in two different laboratories by Saavedra and Axelrod (41) and by Knott *et al.* (42). Both the *in vivo* and *in vitro* conversion of tryptamine to DMT was later demonstrated using rodent brains by Saavedra and Axelrod (43). The enzyme apparently responsible for DMT formation (i.e., indolealkylamine-*N*-methyltransferase) has been isolated from brain tissue (44), rabbit lung (43), and recently from human cerebrospinal fluid (45). In these tissue sources the enzyme's primary methyl donor was identified as *S*-adenosylmethionine. Therefore, since the enzyme, substrate, and cofactor are all present in brain tissue it seems reasonable to assume that the compound (DMT) is not only synthesized *in vivo* but that its presence suggests that the compound may have a specific function in the CNS.

We have previously shown that both the precursor (tryptamine) and the product DMT are located in the synaptosomal fraction of rodent brains (24, 25). It should probably be restated here that the extraction efficiencies for both DMT and tryptamine from biological material using the methylene chloride solvent system was found to be 90+% efficient for both compounds (18). This present study localizes dimethyltryptamine in the vesicles of the neuron. We also find a considerable amount of tryptamine in the vesicular supernatant (S_6). These data are clearly indicated in Table 1. It is, of course, well known that the concentration of any neurotransmitter in the neuronal vesicle is very much greater than its concentration in the corresponding cytosol (46). Consequently, one is forced to conclude that a specific pumping mechanism may be required to maintain this concentration gradient and that considerable energy is required for the process. During the isolation of the subcellular neuronal elements, however, the temperature is maintained at 4°. Considerable leakage of most transmitters occurs at this temperature, presumably because the activity of enzymatic systems, which (1) supply the necessary energy source and (2) the carrier or pumping system, is considerably depressed. This idea is borne out by our finding that at room temperature, with the addition of ATP and Mg^{2+}, transfer of DMT into the vesicles is apparently facilitated (see Experiment 3, Table 1). Since we have not as yet studied the active transport process in detail, this finding must remain a preliminary observation. One further point which should be emphasized from our measurements is the inability of ATP and Mg^{2+} to bring about an increased concentration of tryptamine in the vesicles themselves. From these data it would appear that the DMT is selectively taken up by the neuronal vesicles. Fortunately these measurements were made possible by

the modification of a sensitive glc analytical procedure previously developed in our laboratory (6, 18).

Having localized DMT in the neuronal vesicle, the next logical step was to determine if a specific binding site could be found on synaptosomes for the compound. In order to answer this question the technique of equilibrium dialysis was invoked. As a tissue source for these binding studies, only lysed synaptosomes were utilized. A Scatchard plot (see Fig. 4) of *d*-LSD indicated that a high affinity binding site could be demonstrated on lysed membranes with an approximate K_d of 3 nM. This value is close to the value obtained by both Bennet and Aghajanian (31) and Lovell and Freedman (47). We were able to displace completely bound LSD at a DMT concentration of 1×10^{-5} M. This finding is likewise in agreement with the recent publications of Lovell and Freedman (47). Their data show that the IC_{50} (the amount of ligand necessary to displace 50% of the bound compound) for DMT is approximately 2.2×10^{-6} M. In order for DMT to displace LSD bound at its high affinity site the compound must have a finite affinity for that site. In like manner, we found that a second psychotogen of the indolealkylamine type, *O*-methylbufotenin (5-methoxy-*N*,*N*-dimethyltryptamine) would also completely displace the bound LSD at a concentration of 1×10^{-5} M. Lovell and Freedman report an IC_{50} of 9.7×10^{-7} for this compound. The idea of a "common" psychotogen binding site for the indolealkylamine type of hallucinogens was suggested by Smythies *et al.* in 1970 (32). Our data, even though limited in the number of psychotogens, support this concept (48). After obtaining data on LSD displacement we also felt that in order to give the idea of an endogenous DMT binding site more validity the possible displacement of [^{3}H]-serotonin should be examined. Consequently, the highest affinity 5-HT binding site was chosen as the test site (a complete analysis of 5-HT binding will be the basis for a future publication). Peak binding of 5-HT at this site occurs at approximately 5×10^{-10} M with a K_d of 2.4×10^{-10} **M** (see the Hill plot in Fig. 5). Equilibrium dialysis studies on the synaptosomal membranes obtained by lysis, which have [^{3}H]-serotonin bound at a concentration of 5×10^{-10} M, did not exhibit any displacement of labeled 5-HT at DMT concentrations up to 5×10^{-5} M. These data then would substantiate the idea of a definitive DMT specific binding site.

If DMT is acting as a neuroregulatory agent *in vivo*, one might speculate that DMT binding to synaptosomal membranes would be accompanied by a direct effect on the adenylate cyclase system found in these membranes. The results reported here do, in fact, show that DMT even at very low concentrations, i.e., 5×10^{-10} M, can stimulate cAMP production. This production is shown in Fig. 6 relative to the endogenous control. Several investigators have suggested that a neurotransmitter should stimulate

adenylate cyclase as a consequence of postsynaptic transmitter binding. DMT does satisfy this criterion. It is interesting to note that even at the low concentrations utilized in this study tryptamine appears to inhibit adenylate cyclase activity. Some investigators have proposed that tryptamine does have a neuroregulatory function (38, 39). Consequently, these data seem to support this concept. As a further exercise designed to show the differentiation between the DMT and the serotonin site, experiments utilizing equimolar combinations of DMT, LSD, and 5-HT were carried out. The results of this study indicate that (1) *d*-LSD apparently has a higher affinity for the synaptosomes than does DMT, but even with this higher affinity it does not generate as much cAMP as does DMT, and (2) that combinations of DMT plus serotonin and LSD plus serotonin generate an amount of cAMP consistent with site differentiation.

Since we have shown that DMT and its precursor, tryptamine, may be found in the cerebrospinal fluid of humans (19, 20, 24, 53), presumably having its origin in the brain, and in the vesicle fraction of rodent brains, the question arises as to the possible *in vivo* function of this compound. If the results of this study are viewed in light of the accepted criteria for a neurotransmitter, then DMT appears to meet nearly all of these criteria. For example, as previously stated the necessary synthetic machinery is present in CNS tissue for the synthesis of the compound (44, 45) and as this study shows, a binding site appears to be present with which the compound can interact and as a consequence of this interaction cAMP is produced. The compound seems to be concentrated into neuronal vesicles by an active transport process.

In addition to these findings, as a measure of its electrophysiological activity, Berridge (49) and Berridge and Prince (50) have shown that DMT stimulates fluid secretion from the salivary glands of blowflies, changes the transepithelial and intracellular potentials of the gland, and increases the production of cyclic AMP. The compound if ingested by man (2) or animals (3) at the level of approximately 1 mg/kg body wt produces hallucinations. At this point the degradation of DMT is unclear. Some reports have appeared which suggest that the compound may be metabolized *in vivo* to indoleacetic acid (51, 52).

From a teleological point of view, the data arising from this study would suggest that one possible mode of action of LSD would be its displacement of DMT at the synaptosomal membrane level. Lysergic acid diethylamide exhibits a somewhat lower ability to stimulate adenylate cyclase activity in synaptosomal membranes although it does have a high affinity for the DMT binding site. The consequences of such DMT displacement are at this point a matter of conjecture with respect to normal neuronal activity. We are at present examining the differential production of cAMP and cGMP by these compounds at the DMT binding site.

There are, however, several points which need clarification. Although the compound is found *in vivo* and has central activity, at this point, we do not know whether or not DMT is acting at a postsynaptic receptor site or whether it acts preferentially on a presynaptic reuptake site. In addition, the regional brain distribution of the compound is not known at this time, although the methodology is now available for such studies (18). Almost nothing is known about the electrophysiology of the compound. The compound may also be a type of neuroregulatory agent rather than a putative transmitter. The answers to these questions as well as others which may arise must be found before the exact function of this compound can be identified. At least some of the needed answers may be forthcoming with the synthesis of a high specific activity radiolabeled dimethyltryptamine.

Our identification of DMT as a normal constituent of brain may shed new light on the idea that the compound need only be present to produce schizophrenic symptoms in man (2). Since we have already identified this agent in the cerebrospinal fluid of normal humans (19, 20, 37), then one might propose that it is in fact not the presence of the compound that is disease producing, but it may be elevated levels of the compound. Preliminary experiments in our laboratory have indicated that rats under stress (electrical shock) have higher DMT brain levels than do control animals (54). That is, a threshold level may exist such that an increase in the amount of DMT above this level is psychotoxic, while amounts below this level serve some as yet unknown but normal neurogenic function.

SUMMARY

The psychotomimetic agent dimethyltryptamine (DMT) has been identified as an endogenous compound in the central nervous system of rodents using a sensitive electron capture gas chromatographic technique. DMT along with its proposed precursor, tryptamine, were identified and quantitated as the heptafluorobutyryl derivatives. A specific high affinity binding site on synaptosomal membranes has been proposed for DMT. This proposal is based on equilibrium dialysis experiments which indicate that DMT at a concentration of 1×10^{-5} M will displace *d*-LSD on isolated membranes but will not displace bound serotonin at the same concentration. When DMT interacts with the synaptosomal membranes at a concentration of 5×10^{-10} M, the membrane-bound enzyme adenylate cyclase is stimulated such that adenosine3′,5′-monophosphate (cAMP) is produced at a rate of 100 pmole/min/mg of protein (2.3 times the endogenous rate). It has also been shown that its presumed precursor, tryptamine, inhibits this process. LSD appears to exhibit a high affinity for the proposed DMT binding site but seems to have a low intrinsic activity. From data obtained in this study it has been postulated that DMT may have *in*

vivo activity similar to those proposed for neurotransmitters or other neuroregulatory agents. These data further suggest that at least one mode of action of *d*-LSD may be the displacement of DMT from its binding site on the neuron.

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