EVIDENCE FOR DIMETHYLTRYPTAMINE (DMT) AS A NATURALLY-OCCURRING TRANSMITTER IN MAMMALIAN BRAIN*#

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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 broad classification, we will present data here suggesting that the common hallucinogenic agent, dimethyltryptamine (DMT), is a naturally-occurring neurotransmitter in the central nervous system of both rodents and man. The occurrence of its precursor, tryptamine, in both animal and human brain has been verified by several investigators. One of us (1) using a gas liquid chromatographic technique along with the fluorometric technique of Hess and Udenfriend (2) found nanogram quantities of this compound in human, steer, and dog brain. These findings were later verified by Saavedra and Axelrod using rodent brains (3), and by Knott et al. (4). The in vitro conversion of tryptamine to dimethyltryptamine has been demonstrated by Axelrod (5) using an enzyme derived from rabbit lung and by Mandell and Morgan (6) using an enzyme from rat brain. In both of these systems the enzyme, indolealkylamine-N-methyltransferase, utilized S-adenosylmethionine as the primary methyl donor. Therefore, since the precursor of DMT is found in CNS tissue along with the necessary enzyme system and methyl donor, it seems reasonable to assume that this compound may be synthesized in vivo.

We have recently developed a quantitative and ultra-sensitive method for the detection and identification of several of the neutral and basic biogenic amines containing the indole nucleus including both DMT and tryptamine (7,8). With this method we can clearly identify not only the latter two compounds but also N-methyltryptamine, 5-methoxy-N,N-dimethyltryptamine, 5-methoxytryptamine and bufotenine. These compounds may be detected in the low picogram range with a high degree of accuracy. The method is suitable for the analysis of both tissue and cerebrospinal fluid. The isolated indolealkylamines are derivatized with heptafluorobutyryl imidazole (HFBI) and identified as their N-perfluoroacyl derivatives using gas-liquid-chromatography by direct comparison with known external standards (8). This technique has been successfully utilized by us to detect DMT and its precursor, tryptamine, in isolated synaptic vesicles from rat brain. Synaptosomes were prepared from 200 g Sprague-Dawley male rats, fed and watered ad libitum, essentially by a slight modification of the procedure described by Cotman (9). After obtaining the synaptosome band we lysed the synaptosomes by resuspension in hypotonic (5mM Tris-HCl, pH 8.3) buffer. The lysed synaptosomes from this procedure were utilized later for binding studies while the neuronal vesicles were isolated by a modification of the method described by Morgan et al. (10). The highly purified vesicles were then extracted with methylene chloride and the extract derivitized with HFBI and then analyzed on a gas liquid chromatograph equipped with a ^{63}Ni electron capture detector. The results of this experiment are shown in Figures 1-3. The first figure is a calibration curve showing the retention times and relative-detector response of the chromatograph to 200 picograms of HFB-tryptamine and HFB-dimethyltryptamine (8). Figure 2 is the chromatographic record of the extracted and derivatized vesicles from six rats. This figure demonstrates the appearance of well-defined peaks at 6.5 minutes and 12.5 minutes. These peaks correspond to the exact retention times of the pure derivatized external standards shown in Figure 1. From our calibration curves these peak areas represent 170 pg of DMT and 110 pg of tryptamine, respectively. Figure 3 is the same derivatized material as shown in Figure 2 except it has been "spiked" with 100 pg of the DMT external standard as an additional confirmatory measure. This figure illustrates that the retention times are indeed identical and that the new peak area is exactly as expected. Based on the weight of vesicular protein the amount of tryptamine is 6.6 ng/mg and 10.0 ng/mg of DMT. Protein was determined by the method of Lowry

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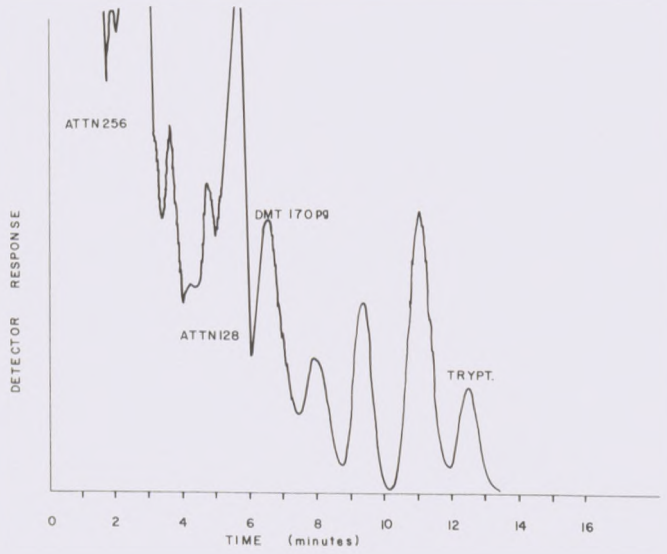

Figure 1

Gas chromatogram of the perfluoroacyl derivatives of tryptamine and dimethyltryptamine. These compounds were derivatized with heptafluorobutyryl imidazole and isolated and purified according to reference (7). The glc used was a Hewlett-Packard 5700A equipped with a ^{63}Ni electron capture detector. The column was a 1.8m x 2mm 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 derivatized standard were injected onto the column. Instrument conditions were as follows: injection block temperature — 250°, oven temperature: 175°, gas flow (95% argon — 5% methane) — 50 ml/min, detector temperature, 300°. The retention times were 6.5 minutes for DMT and 12.5 minutes for tryptamine. The extraction efficiency of methylene chloride for tryptamine was found experimentally to be greater than 98 per cent.

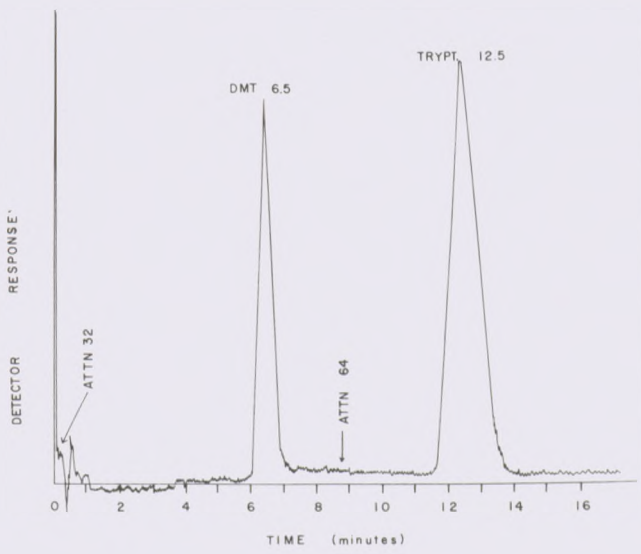

Figure 2

This figure depicts the chromatogram of a derivatized methylene chloride extract of isolated synaptic vesicles from six rat brains. Chromatographic conditions are the same as those shown in Figure 1. The peaks occurring at 6.5 and 12.5 minutes have retention times identical to those for the pure DMT and tryptamine external standards. These same compounds were also separated and identified on both 1.8m and 3.6m columns packed with a 5% SE-30 liquid phase. Although the overall results were identical the OV-17 system gave superior results. Calibration curves for the external standards were linear over the entire range of concentrations detected.

(11). In addition to the identification of DMT and its precursor in synaptic vesicles we have also identified these compounds in human cerebrospinal fluid (12). The same gas chromatographic procedure was utilized. The spinal fluid was obtained by a standard lumbar puncture from patients undergoing air-encephalograms or other clinical laboratory procedures. Complete details of the human analytical study will be published elsewhere.

In order to ascertain whether or not DMT is bound to the synaptosomal membrane, an indirect analysis was carried out using the technique of equilibrium dialysis. For this study all-Teflon dialysis cells were used as an integral part of the Kontron-Diapack (Kontron) equilibrium dialysis system. Lysed synaptosomes were prepared from rat brains as described. Using this technique, from Scatchard (13) plots it was found that radiolabeled d-lysergic acid diethylamide (LSD) had a binding constant (K_d) of 2.9×10^{-9} M. This value is in agreement with both the value reported by Farrow and Van Vanakis (14) and the value reported recently by Bennett and Snyder (15). It was found that bound LSD could be completely displaced from its high affinity binding site by 1×10^{-5} M dimethyltryptamine. It was also found that 5-methoxy-N,N-dimethyltryptamine (OMB) could also displace LSD from the synaptosomes (16). Several other laboratories have reported similar results (14,15). From such data it has been suggested that a "common" high affinity hallucinogenic binding site is present in brain tissue (16). Several investigators have suggested that this site is the serotonin (5-HT) binding site (17-20). However, this assertion is still somewhat controversial since there must be more than one kind of 5-HT binding site (e.g. presynaptic reuptake and postsynaptic depolarizing etc.). In fact,

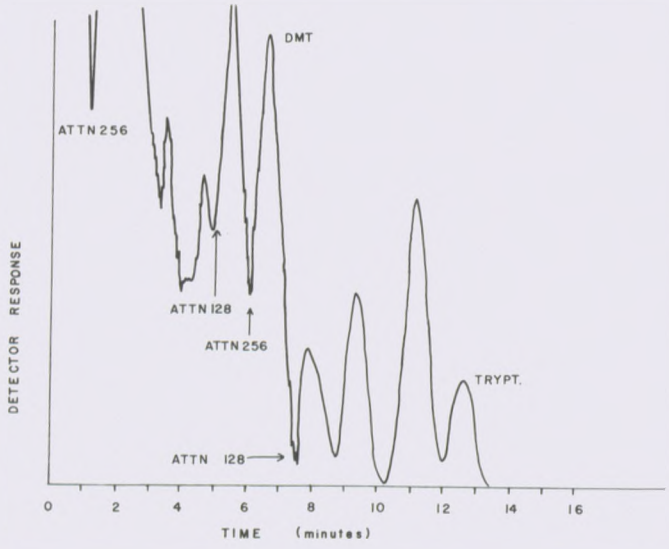

Figure 3

A known amount (100 pg) of DMT was added to the heptafluorobutyryl, derivatized vesicle extract in toluene. Three microliters were again injected into the instrument. No change was noted in the chromatogram except that which would be expected on a quantitative basis. The change in DMT peak area is exactly what would be expected from the calibration curve. The chromatogram further shows that the retention time is identical to that shown in Figure 2. The "spiked" sample also does not reveal any shoulders or distortions in the DMT peak. The instrumental parameters in this experiment were again identical to those reported in Figure 1.

using affinity chromatography one group (21) has isolated two distinct 5-HT binding proteins from rat brain while another (using the same general technique) has found six (22). Consequently, the data obtained by us and by several other investigators does indicate that DMT can bind to specific sites in CNS tissue.

We now know that 1) the synthetic enzymes and substrates are present in CNS tissue for the production of DMT, and 2) a binding site is present to react with the compound, and 3) the compound may be found in both human cerebrospinal fluid and isolated synaptic vesicles from rat brain tissue. Further evidence to suggest DMT as a candidate for putative transmitter status is the biological and electrophysiological activity of the molecule. In this respect we have known for a considerable time that DMT is a psychotomimetic drug which produces hallucinations when given to man. In addition to this gross measure of its activity Berridge (23), and Berridge and Prince (17), have shown that DMT stimulates fluid secretion from the salivary glands of blow-flies, changes the transepithelial and intracellular potentials of the gland and increases the production of 3′5′-adenosine monophosphate (cylic AMP). With respect to cyclic AMP production we have determined in this laboratory that when DMT binds to the lysed synaptosome preparation, 5×10^{-13} moles of DMT will generate 71.0 pmoles of the cyclic nucleotide per minute per milligram of synaptosomes. These determinations were made using the method of Nathanson and Greengard (24). When all the foregoing points are considered the only parameter remaining necessary to classify this compound as a transmitter concerns its metabolism at the synapse. One of many possibilities suggests that perhaps the compound is demethylated and converted to indoleacetic acid in much the same way that 5-methoxy-N,N-dimethyltryptamine is metabolized as shown by Agurell et al. (25) to 5-methoxyindoleacetic acid. However, this latter point remains to be proven.

The idea that DMT is a neurotransmitter conjures up several exciting possibilities as to why (from a teleological point of view) an animal should have such a naturally occurring compound that, in sufficiently high concentration, can produce hallucinations. Several investigators have also implicated this compound as the possible cause for certain psychiatric disorders such as schizophrenia (26-28). The answer to these questions as well as those perhaps not yet even formulated will have to await further experimentation.

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