Psychotomimetic N-Methylated Tryptamines: Formation in Brain in vivo and in vitro

Abstract. *The use of a sensitive enzymatic assay demonstrates that tryptamine occurs normally in rat brain. Intracisternal administration of [^{14}C]tryptamine results in the formation of* N*-methyl- and dimethyltryptamine (a psychotomimetic compound) in the rat brain. An enzyme that converts tryptamine and* N*-methyltryptamine to* N*-methyl- and dimethyltryptamine was found to be present in rat and human brain. The* N*-methylation of tryptamine was inhibited by normally occurring compounds present in rat brain.*

Dimethyltryptamine has been shown to produce psychotomimetic effects in man (*1*). An enzyme in the rabbit lung that can form dimethyltryptamine from tryptamine and *N*-methyltryptamine has been described (*2*). The administration of L-tryptophan, the amino acid precursor of tryptamine, and L-methionine to schizophrenic patients resulted in intensification of the symptomatology (*3*). Dimethyltryptamine has also been reported to be present in the urine of schizophrenic subjects treated with monoamine oxidase (MAO) inhibitors (*4*). We now report the normal occurrence of tryptamine in brain as well as its conversion to mono- and dimethyltryptamine in vivo and in vitro.

Previous attempts to detect the normal occurrence of tryptamine in the rat brain have been unsuccessful (*5*). Small amounts of tryptamine have been reported in bovine brain (*6*). Using rabbit lung *N*-methyltransferase, an enzyme that can transfer the methyl group of *S*-adenosyl[methyl-^{14}C]methionine to tryptamine, we have developed a specific and sensitive assay for measuring tryptamine in brain and other tissues (*7*). In this procedure, endogenous tryptamine is separated from interfering substances by extraction with a mixture of toluene and isoamyl alcohol (97 : 3) and is enzymatically converted to *N*-[methyl-^{14}C]-methyltryptamine with a preparation of rabbit lung *N*-methyltransferase and ^{14}C-labeled adenosylmethionine. With this procedure tryptamine was found to be normally occurring in the rat brain in small amounts (20 ng per gram of tissue).

The presence of tryptamine in the brain prompted a study of the possible conversion of this amine to methylated tryptamine in vivo. Five Sprague-Dawley rats, weighing 250 to 300 g, were first treated with the MAO inhibitor pheniphrazine. The rats were lightly anesthetized with ether and injected intracisternally (*8*) with 10 μc of [^{14}C]tryptamine (*9*) diluted in 20 μl of saline. The rats were killed 30 minutes later, and the brains were immediately removed and homogenized in 10 ml of 0.1*N* hydrochloric acid. After centrifugation of the homogenate, the supernatant fraction was made alkaline with 1*N* NaOH, and extracted into two volumes of toluene containing 3 percent isoamyl alcohol. The organic extracts were dried under an atmosphere of nitrogen, and the residue was taken up in 200 μl of ethanol. A 5-μl portion (40,000 to 50,000 count/min) was subjected to bidimensional thin-layer chromatography first with solvent A (*10*), and then with solvent B. This system sharply separated tryptamine, *N*-methyltryptamine, and dimethyltryptamine (Fig. 1). A larger and a smaller radioactive peak, corresponding to the R_F of authentic *N*-methyltryptamine and dimethyltryptamine, respectively, were present. The amount of the *N*-methylated compounds represented between 1 and 2 percent of the radioactive products extracted from the brain. The apparent ^{14}C-labeled *N*-methyl- and dimethyltryptamine also had the same R_F values as the authentic indoles, in three additional solvent systems (C, D, and E) (*10*).

The brain contains *O*- and *N*-methylating enzymes for catecholamines, histamine (*11*), and serotonin (*12*). The serotonin *N*-methylating enzyme in the brain was also found to form a metabolite with tryptamine, but its identity was not established (*12*). Initial attempts to detect the enzymatic *N*-methylation of tryptamine in the rat brain were unsuccessful, because of large amounts of radioactivity extracted from brain tissue in the absence of substrate, the presence of an enzyme in the brain which converts adenosyl[methyl-^{14}C]methionine to methanol (*13*), and normally occurring inhibitors. These problems were overcome by dialyzing the brain preparation, by using a less polar solvent (toluene, 3 percent isoamyl alcohol) for extraction of the ^{14}C-*N*-methylated tryptamine, and by evaporating the organic extract of the enzymatic reaction to eliminate the volatile [^{14}C]methanol (Table 1). Rats were killed, and their brains were rapidly removed, immediately homogenized in 10 ml of ice-cold 0.01*M* sodium phosphate buffer, *p*H 7.9, and centrifuged at 100,000*g* for 40 minutes. The supernatant fraction was dialyzed against 0.01*M* sodium phosphate buffer, *p*H 7.9, for 12 hours. A 200-μl portion of the dialyzed supernatant was incubated with 5 nmole of adenosyl[methyl-^{14}C]-methionine (*14*) and 0.01*M* sodium phosphate buffer, *p*H 7.9, in a volume of 0.5 ml, with and without the addition of 200 μg of tryptamine. After incubation for 90 minutes at 37°C, 1 ml of 0.5*M* borate buffer, *p*H 10, was added, and the radioactive product was extracted with 6 ml of toluene containing 3 percent isoamyl alcohol. The extract was shaken for 30 seconds and centrifuged; a 5-ml portion of the organic phase was transferred to a counting vial and the solvent was evaporated to dryness at 80°C in a chromatography oven. The residue was taken up in 2 ml of

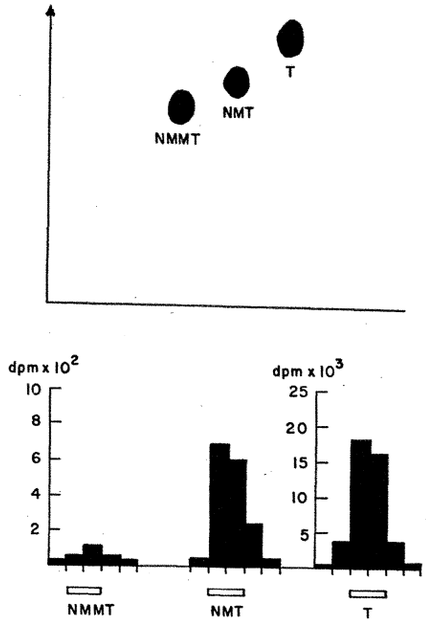

Fig. 1. Chromatographic identification of the formation of *N*-methylated metabolites of tryptamine in the intact rat brain. The solvent systems used were A (*10*) for the first dimension (vertical in the figure), and B for the second dimension (horizontal in the figure). See text; *NMMT*, dimethyltryptamine; *NMT*, *N*-methyltryptamine; *T*, tryptamine; dpm, disintegrations per minute.

Table 1. Enzymatic *N*-methylation of tryptamine in rat and human brain. Brain supernatant fractions incubated with adenosyl[methyl-^{14}C]methionine, with and without tryptamine or *N*-methyltryptamine, were extracted and evaporated as described in the text.

Treatment		Substrate added	Enzymatic activity	
Brain supernatant	Organic extract		Disintegrations per minute per 90 minutes of incubation	Picomoles per gram of tissue per hour
		Rat brain		
None	None	None	3020	
None	Evaporated	None	199	
None	Evaporated	Tryptamine	341	40
Dialyzed	Evaporated	None	169	
Dialyzed	Evaporated	Tryptamine	1350	350
Dialyzed	Evaporated	*N*-Methyltryptamine	995	250
		Human brain		
Dialyzed	Evaporated	None	132	
Dialyzed	Evaporated	Tryptamine	1650	450
Dialyzed	Evaporated	*N*-Methyltryptamine	1320	360

ethanol and 10 ml of phosphor, and the radioactivity was counted. The addition of tryptamine to the incubation mixture resulted in the formation of a radioactive product (Table 1). Heated enzyme was not active. For identification of the product, larger amounts of brain extracts and substrates were incubated and extracted as above. The solvent extract was evaporated at room temperature under nitrogen atmosphere and chromatographed in systems A, B, C, D, and E (*10*). Radioactive peaks isographic with authentic *N*-methyltryptamine and smaller peaks isographic with authentic dimethyltryptamine were found in all of the solvents used. The conversion of *N*-methyltryptamine to dimethyltryptamine was also confirmed with the use of *N*-methyltryptamine as a substrate. The radioactive product formed was identified as dimethyltryptamine in the same solvent systems as above. Human brain biopsy specimens from temporal lobe, obtained from neurosurgical operations, were also assayed for their enzymatic *N*-methylating activity. All human brain samples were capable of *N*-methylating tryptamine to *N*-methyltryptamine.

The normal occurrence of tryptamine in the brain, as well as its capacity to form a psychotomimetic *N*-methylated metabolite has implications to pharmacology, psychiatry, and biochemistry. The presence of an inhibitor of the *N*-methylating enzyme suggests that its activity might be regulated by endogenous compounds in the brain. The nature of the normally occurring inhibitor of the methylating enzyme has not been established. However, serotonin, catecholamines, and histamine could inhibit the tryptamine methylation since they are substrates for the nonspecific methylating enzyme (*2*) as well as other *S*-adenosylmethionine–requiring enzymes (*11, 12*). These biogenic amines are highly localized in different regions of the brain (*15*). In addition, *S*-adenosylhomocysteine, a compound normally occurring in the brain, has recently been reported to produce inhibition of several methyltransferases (*16*). Thus it is possible that changes in levels of serotonin, catecholamines, histamine, or *S*-adenosylmethionine might affect the *N*-methylation of tryptamine in specific brain areas.

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10. The identification of the ^{14}C-methylated tryptamine was made by thin-layer chromatography on Eastman chromagram sheets of silica gel, 100 μm in thickness. All sheets were activated by heating for 30 minutes at 90°C in an oven, immediately before being used. Solvents used: A is *n*-butanol, acetic acid, water (12 : 3 : 5); B is isopropanol, 10 percent ammonium hydroxide, water (200 : 10 : 20); C is methyl acetate, isopropanol, 10 percent ammonium hydroxide (45 : 35 : 20); D is acetone, ammonium hydroxide (90 : 1); and E is toluene, acetic acid, ethyl acetate, water (80 : 40 : 20 : 5).
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1-Methyladenine Biosynthesis in Starfish Ovary: Action of Gonad-Stimulating Hormone in Methylation

Abstract. *Gonad-stimulating substance, a hormonal peptide released from the nervous system of starfishes, acts on the ovary to produce 1-methyladenine, an inducer of oocyte maturation. Addition of methionine to the incubation mixture of ovarian fragments and gonad-stimulating substance enhanced the production of 1-methyladenine, whereas ethionine inhibited it. Incubation of ovarian tissue with methionine alone failed to produce 1-methyladenine. Use of a radioactive label showed that methionine is a methyl donor in the biosynthesis of 1-methyladenine, suggesting that gonad-stimulating substance is involved in the methylation process.*

Oocyte maturation and spawning in the starfish are triggered by a hormonal peptide, gonad-stimulating substance (GSS), which is released from nervous tissue (*1*). This peptide consists of about 22 amino acids and has a molecular weight of 2100 (*2*). The action of GSS is indirect (*3*); it acts on the ovary to produce maturation-inducing substance (MIS), which is a direct inducer of oocyte maturation and spawning in starfishes. The substance MIS has been isolated and identified as 1-methyladenine (*4*). Thus the role of GSS in such reproductive phenomena seems to be to synthesize 1-methyladenine, which is produced by the follicles around the oocytes (*5*). Since methionine—in its active form, *S*-adenosylmethionine (*6*)—donates the methyl