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Methyltetrahydrofolic Acid Mediates N- and O-Methylation of Biogenic Amines

Abstract. *A variety of mammalian and avian tissues* N- *and* O-*methylate indoleamines and phenylethylamines, with methyltetrahydrofolic acid as the methyl donor. Because it is considerably more efficient than* S-*adenosylmethionine, methyltetrahydrofolic acid may be the natural methyl donor in this reaction. With methyltetrahydrofolic acid, serotonin is* O-*methylated to 5-methoxytryptamine, a novel indoleamine in mammalian brain.*

The *N*-methylation of biogenic amines may play a major role in certain mental illnesses (*1*). Axelrod described an enzyme that *N*-methylated a variety of biogenic amines, including indoleamines, but the enzyme could not be demonstrated in tissues other than the rabbit lung (*2*). It has been reported that chick and human brains (*3*) and a variety of dialyzed mammalian tissues (*4*) contain enzymes that can *N*-methylate indoleamines. All of these enzymes use *S*-adenosylmethionine (AMe) as a methyl donor and, except for the rabbit lung enzyme, their activity is low (*2–4*). Laduron (*5*) observed that dopamine can be *N*-methylated to epinine with methyltetrahydrofolic acid (MTHF) as the methyl donor. We now report the *N*- and *O*-methylation of several biogenic amines by extracts of mammalian and avian tissues. The primary methyl donor appears to be MTHF.

Tissues from male rats (150 to 200 g), male rabbits (1.5 kg), and 5-day-old Leghorn chicks (mixed sex) were homogenized in ten volumes of 0.005*M* sodium phosphate buffer (*p*H 7.9), and the homogenates were centrifuged at 100,000*g* for 60 minutes. We dialyzed some supernatant preparations for 12 hours against 100 to 200 volumes of the same sodium phosphate buffer. Incubation mixtures for the enzyme assay contained, at final concentrations, sodium phosphate buffer, *p*H 7.9, 0.005*M*; amine substrate, 5 m*M*; [^{14}C]MTHF (50 mc/mmole; Amersham), 1 μM; or [^{14}C]AMe (50 mc/mmole; New England Nuclear), 1 μM; together with tissue enzyme (0.5 to 2.0 mg of protein per milliliter) in a final volume of 0.5 ml. The mixture was incubated for 30 to 60 minutes at 37°C; 1 ml of 0.5*M* borate buffer, *p*H 10, was added and the mixture was added to 6 ml of an organic solvent, selected on the basis of the amine substrate. After shaking the mixture for 10 minutes, it was 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. We dissolved the residue in 2 ml of ethanol, added 10 ml of toluene phospor, and counted the radioactivity. Controls, consisting of incubation mixtures that lacked substrate amine, showed radioactivity similar to that of the blanks obtained by heating the enzyme preparation. Enzyme activity was linear for at least 60 minutes. Methylated products were identified by thin-layer chromatography in three solvent systems (*6*). In all cases the radioactive peaks coincided with *N*- or *O*-methylated forms of the amines.

As reported, both undialyzed and dialyzed supernatant preparations of rabbit lung methylated serotonin and tyramine with AMe as the methyl donor (*2*) (Table 1). Supernatant preparations from undialyzed rat lung, rat brain, and rabbit brain failed to significantly methylate either substrate with AMe (*7*), while undialyzed chick brain preparations methylated amines with AMe (*3*). However, except for the enzymes from rabbit lung, which methylated amines as efficiently when dialyzed or not dialyzed, methylation by enzymes from other tissues was greatly enhanced by dialysis.

Dialyzed supernatant preparations of rabbit lung methylated serotonin and tyramine 4 to 6 times more efficiently with AMe than with MTHF. In all other tissues, tyramine methylation was considerably greater with MTHF than with AMe and we did not detect any serotonin methylation with AMe. In all tissues examined, serotonin was methylated with MTHF 1.5 to 3 times more efficiently than was tyramine.

The MTHF requiring methylating enzyme was partially purified from rat brain by ammonium sulfate fractionations and chromatography (*8*). The materials that precipitated when the solutions were 30 to 45 percent and 45 to 60 percent saturated with ammonium sulfate had similar activities

Table 1. Species and tissue distribution of methyltransferase activity. Tissues were homogenized in ten volumes of 5 μM sodium phosphate buffer (*p*H 7.9). After dialysis, the solutions were centrifuged at 100,000*g* and the supernatant was assayed for enzyme activity. Serotonin (5 m*M*) (S) or tyramine (5 m*M*) (T) were substrates and *S*-adenosylmethionine (1 μM) or 5-methyltetrahydrofolic acid (1 μM) were methyl donors. Data are presented as the mean of three experiments whose results varied less than 20 percent. Enzyme activity is expressed as picomoles of methyl group added to the substrate per milligram of protein in 1 hour.

Tissue	AMe			MTHF			Ratio of enzyme activity with AMe to activity with MTHF	
	S	T	S/T	S	T	S/T	S	T
Rabbit lung	32.0	24.0	1.33	8.0	4.0	2.0	4.0	6.0
Rabbit brain	0	0.05	0	2.6	1.4	1.9	0	0.04
Rabbit liver	0	0.2	0	2.1	1.2	1.7	0	0.08
Rat brain	0	0.4	0	3.0	1.3	2.3	0	0.31
Rat liver	0	1.2	0	4.3	1.4	3.1	0	0.90
Rat lung	0	0.35	0	4.0	1.3	3.1	0	0.27
Rat heart	0	0.4	0	8.0	3.0	2.7	0	0.14
Chick brain	0	1.0	0	6.0	4.0	1.5	0	0.25
Chick heart	0	2.0	0	26.0	11.0	2.3	0	0.18

with AMe and MTHF as methyl donors and with tyramine and tryptamine as substrates. The material that precipitated at 75 percent saturation and the resulting supernatant solution failed to methylate tyramine or tryptamine with AMe but methylated these amines vigorously with MTHF. The specific activity of the final supernatant fraction after 75 percent ammonium sulfate saturation was four to five times greater than the activity of the original homogenate with tyramine and tryptamine as amine substrates and MTHF as methyl donor. This fraction retained 40 percent of the enzyme activity of the original tissue homogenate and was further purified by a negative adsorption of alumina-C γ gel (Calbiochem), resulting in a further four- to fivefold increase in specific activity for methylation of tyramine and tryptamine with MTHF, but still with no methylating activity in the presence of AMe. Thus this two-step procedure resulted in a 20-fold purification of the enzyme with 25 percent recovery of total activity.

The Michaelis constant of the partially purified enzyme for MTHF was 10 μM and that for serotonin was 0.2 mM. The affinity constant for tyramine was about 1 mM with either MTHF or AMe as methyl donor (*8*). The purified enzyme was most active toward serotonin, *N*-methylserotonin, and bufotenin which were methylated about twice as efficiently as tyramine or tryptamine (Table 2). Octopamine, 5-methoxytryptamine, and β-phenylethylamine were somewhat less easily methylated than tyramine and tryptamine. The relative ease of methylation of amine substances was the same for the purified enzyme as for the original supernatant of rat brain homogenate.

We used thin-layer chromatography to analyze the products produced when the amines were incubated with partially purified or initial supernatant preparations. There were two distinct types of methylated metabolites. With tryptamine as substrate, the labeled product was predominantly *N*-methyltryptamine with only small amounts of material migrating like *N,N*-dimethyltryptamine. However, *N*-methylation of serotonin appeared to represent only a minor pathway, with the bulk of radiolabeled product present as 5-methoxytryptamine. To confirm the existence of *O*-methylation of 5-hydroxyindoles, a reaction previously described only for melatonin synthesis in the pineal gland (*9*), we tested bufotenin as a possible substrate for methylation. Bufotenin, the *N,N*-dimethylated derivative of serotonin, is not likely to be further *N*-methylated. However, bufotenin was almost as easily methylated as serotonin (Table 2). Moreover, thin-layer chromatographic analysis revealed that the product of bufotenin methylation with MTHF was *N,N*-dimethyl-5-methoxytryptamine. The small amounts of radioactivity migrating in the vicinity of bufotenin might conceivably represent methyl exchange with [^{14}C]-MTHF.

Table 2. Substrate specificity of partially purified rat brain methyltransferase with MTHF as methyl donor. The enzyme source is the supernatant fraction after 75 percent ammonium sulfate precipitation. Enzymatic activity is expressed as picomoles of methyl group added to the substrate per milligram of protein in 1 hour; (1) extracted with a mixture of toluene and isoamyl alcohol (3 : 2) and dried overnight at 80°C in a chromatography oven; (2) extracted with a toluene isoamyl alcohol mixture (97 : 3) and dried in oven; (3) extracted with isoamyl alcohol and dried in oven; (4) extracted as in (2) but counted with no previous drying procedure because of the volatility of the product; S.A., specific activity; E.P., extraction procedure.

Substrate	S.A.	E.P.
Tyramine	4.4	1
Tryptamine	4.3	2
Serotonin	8.8	3
β-Phenylethylamine	2.0	4
Octopamine	2.7	1
N-Methyltryptamine	3.4	2
N-Methylserotonin	7.2	3
Desmethylimipramine	0.0	3
5-Methoxytryptamine	2.0	2
Bufotenin (*N,N*-dimethylserotonin)	8.4	3

We interpret the results of our studies of the influence of AMe on MTHF mediated methylation as evidence in favor of distinct enzymes for methyl donation by AMe and MTHF, respectively. The presence of AMe in concentrations ranging from $5 \times 10^{-6} M$ to $10^{-4} M$ did not alter MTHF-mediated methylation of serotonin by supernatant preparations of rat liver. The failure of serotonin to function as a substrate with AMe also suggests the existence of two enzymes with different methyl donors. The process of *O*-methylation might require MTHF, while MTHF and AMe could both serve as donors for *N*-methylation.

In most tissues the enzyme is much more active with MTHF than with AMe, and so MTHF may be the normal methyl donor. Previously, AMe was regarded as a universal methyl donor, especially in methylations of biogenic amines. Our findings and those of Laduron (*5*) indicate that MTHF may be an important biological methyl donor. Also, MTHF can serve as the methyl donor for histamine methyltransferase from rat brain and hydroxyindole-*N*-methyltransferase from bovine pineal, although in both cases AMe is about 50 times as efficient a methyl donor (*8*).

Our demonstration of methylation of biogenic amines by MTHF may have bearing upon amine methylation theories of mental illness. Although the enzyme has broad substrate specificity, serotonin, which is widely implicated in mental illness, is the best substrate. Enzymatic activity is greatest in tissues other than the brain. One may suggest a physiological role for this enzyme since there are significant amounts of 5-methoxytryptamine in mammalian hypothalamus (*10*). The enzymatic formation of 5-methoxy-*N,N*-dimethyltryptamine is of interest, because this compound appears to be a potent psychedelic agent (*11*).

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