A NOTE CONCERNING THE FATE OF THE 4-METHOXYL GROUP IN 3, 4-DIMETHOXYPHENETHYLAMINE (DMPEA)

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The recent controversy over the identity and significance of the "pink spot" in the urine of schizophrenics (Bourdillon, 1965; Wagner, 1966) has led to intense research on O-methylation and demethylation, especially of compounds related to 3, 4-dimethoxyphenethylamine (DMPEA). We have studied demethylation of DMPEA directly by measurement of expired $^{14}CO_2$ from 3-$^{14}CH_3O$, 4-$^{14}CH_3O$, and 8-^{14}C labeled DMPEA, and found that the conversion to $^{14}CO_2$ of the 4-methyl group is approximately 15-fold that of the 3- or 8-labeled compound.

DMPEA has been shown not to be psychotogenic *per se* by Hollister and Friedhoff, (1966) and Shulgin, *et al*, (1966) so its role as an endogenous causal agent in schizophrenia seems unlikely. Since DMPEA may be an intermediate in catecholamine metabolism, its significance in regard to psychosis might be revealed by investigation of its biosynthetic and degradative pathways. One of the principle mechanisms of catecholamine inactivation is the process of O-methylation. Our interest has been directed to the 4-position for several reasons. Enzymatic reactions are known in mammalian tissues for the methylation of a hydroxyl group located at this site (Kuehl *et al*, 1964). In addition to DMPEA, 4-methoxyphenethylamine has been reported as an abnormal component in the urine of schizophrenics by Sen and McGeer (1964). Some half-dozen one ring psychotomimetics are known to be more effective than mescaline in human intoxication and all without exception, as with mescaline itself, are substituted with an alkoxy group in the four position (Shulgin,

1964). In the testing of a variety of such substituted compounds in animals, Ernst (1962) and Michaux and Verly (1963) have found that a methoxyl in the 4-position is essential for retention of pharmacologic activity.

A number of workers (Daly et al, 1960; Friedhoff and Van Winkle, 1963; Kuehl, 1964) have studied methylation and demethylation of a variety of natural amines, their metabolites and analogues, in the 3 and 4 positions, both in vitro and in vivo. In general, their results and conclusions indicate that both the 3 and 4 positions are O-methylated, but that by either preferential methylation or demethylation, the resulting quantity of 4-methylated compounds is about one-tenth of the 3-methylated counterparts. Schweitzer and Friedhoff (1966) in studying the metabolism of 8-^{14}C-DMPEA in the rat found that 6.2% of the metabolites were 4-demethylated compounds; they recovered 67% of the injected ^{14}C in the urine in the first 2 1/2 hours after injection.

The evidence is strong that 4-demethylation of DMPEA occurs in preference to 3-demethylation. We decided to test this directly by labeling DMPEA separately in the 3- and in the 4- methoxy positions, with ^{14}C, and measuring the specific activity of the expired $^{14}CO_2$ when the compounds are injected into rats.

MATERIALS AND METHODS

The 4-$^{14}CH_3O$ labelled DMPEA was synthesized from the potassium salt of vanillin. A solution of this salt in DMSO was treated with $^{14}CH_3I$ (New England Nuclear Co.), and the resulting 4-$^{14}CH_3O$-labelled veratrylaldehyde was converted, by reaction with nitromethane, to the corresponding nitrostyrene. Conventional reduction ($LiAlH_4$) yielded 4-$^{14}CH_3O$ labelled DMPEA with a specific activity of 0.5 mCi/mM and an overall yield of 40%. The employment of potassium iso-vanallate similarly led to the 3-labelled counterpart. The 8-labelled DMPEA was obtained from New England Nuclear Co.

Each experiment consisted of injection of approximately 4 mg. (10 μCi) of labelled DMPEA intravenously (tail vein) to male Buffalo rats. Specific activities and amounts injected (and animal weights) are shown in Table 1. Two rats were injected with each compound.

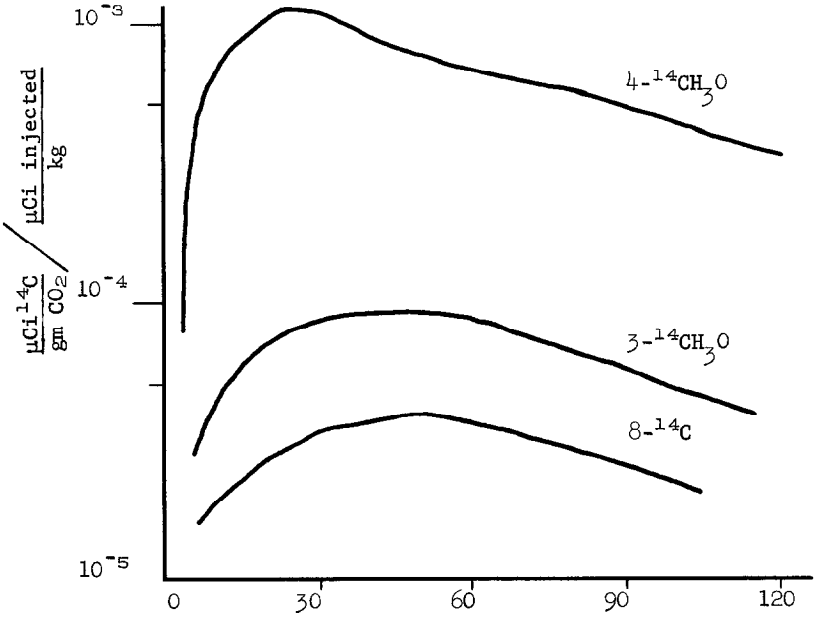
Immediately after injection of the labelled DMPEA, each rat was placed in a 2-liter metabolism cage. Air was continuously evacuated from the cage and passed through a 400 cc. flow-through ionization chamber, and an infrared detector cell, for the continuous monitoring of $^{14}CO_2$ and CO_2 production, respectively. The outputs from the ionization chamber and IR detector were suitably amplified and fed to a multi-channel, continuously recording potentiometer. From the ratio of the signals obtained, and previously determined calibration constants, the specific activity of the $^{14}CO_2$ in the expired air could be continuously determined (Tolbert et al, 1956). By determining the CO_2 production rate and integrating under the curve of $^{14}CO_2$ specific activity vs. time, the amount of $^{14}CO_2$ expired during any interval of time could be obtained. Excretion half-times for $^{14}CO_2$ were obtained from the slopes of these curves when plotted on semi-logarithmic paper.

RESULTS AND DISCUSSION

A continuous record of expired $^{14}CO_2$ on a log scale for three animals each injected with one of the labelled compounds is shown in Fig. 1. ^{14}C appears in the breath within 5 minutes after injection. When the ^{14}C was in the 4-methoxy position, $^{14}CO_2$ production rose rapidly and reached a peak at 25 minutes and declined thereafter with a half-time of approximately 50 minutes. When the label was in the 3-methoxy position, the maximum was more poorly defined, between 30 and 60 minutes; the amount expired was very small, near the lower limit of sensitivity of the instrument. The 8-^{14}C labelled DMPEA produced only barely detectable levels of $^{14}CO_2$.

The curve of expired $^{14}CO_2$ vs. time was integrated with a planimeter over the 2 hour period of the experiment, and the amount of $^{14}CO_2$ expired was calculated as a percent of the amount injected. The results for each animal are shown in Table 1. The amount of $^{14}CO_2$ arising from the 4-$^{14}CH_3O$ of DMPEA was almost 15 times as much as from the 3-$^{14}CH_3O$ counterpart. The 9.6% of $^{14}CO_2$ from the 4 position is quite compatible with the results of Schweitzer and

Figure 1



Friedhoff, who found that 6.2% of the isolated DMPEA metabolities were 4-demethylated.

Table 1

Compound Administered	Animal Weight (gm)	% Administered Dose Excreted as $^{14}CO_2$ in 120 min.	Average %
DMPEA-4-$^{14}CH_3O$	354	10.45%	9.56%
(8.9 μCi, 3.8 mg DMPEA)	340	8.66%	
DMPEA-3-$^{14}CH_3O$	338	0.71%	0.66%
(8.7 μCi, 3.7 mg DMPEA)	330	0.61%	
DMPEA-8-^{14}C	365	< 0.45%	< 0.45%
(9 μCi, < 1 mg DMPEA)	412	< 0.45%	

Kuehl et al (1964) found that with purified rat liver catechol O-methyl transferase, dopamine is converted to both 3-hydroxy-4-methoxyphenethylamine and 4-hydroxy-3-methoxyphenethylamine in a ratio of 4:1, but no DMPEA was detectable. It might be supposed, then, that the 3-methylated products are produced in lesser abundance than the 4-methylated ones, because of this apparently greater 4-methylation of dopamine. However, the work reported here and by Schweitzer and Friedhoff (1966) indicate that the 4 position of DMPEA is actively demethylated. Thus it would appear that an important pathway in the metabolism of catecholamines may be 4-methylation as well as 3-methylation, followed by 4-demethylation. Because of the apparent importance of the 4-methoxy component in known psychotogens, a defect in 4-demethylation should be examined as a possible enzymatic deficiency in schizophrenia.

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