PSYCHOPHARMACOLOGY COMMUNICATIONS, 2(3), 199-206 (1976)

QUANTITATIVE MEASUREMENT OF DEMETHYLATION OF ^{14}C-METHOXYL LABELED DMPEA AND TMA-2 IN RATS

T. Sargent III, A. T. Shulgin and N. Kusubov
Donner Laboratory, University of California
Berkeley, California 94720

Abstract

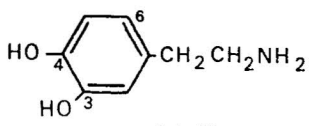
Some reports have suggested that methylation and demethylation of compounds related to 6-hydroxydopamine may be involved in endogenous mental disorder. We report the synthesis of 3,4-dimethoxyphenethylamine (DMPEA) and 2,4,5-trimethoxyphenylisopropylamine (TMA-2) with each methoxyl group separately labeled with ^{14}C. The rate and percent demethylation of these two compounds, with five labeled positions, were determined in the rat. The results suggest that TMA-2 might be metabolized to a hydroquinone *in vivo*; a similar metabolic intermediate of the psychoactive compound DOM is known to give rise *in vitro* to an indole.

Introduction

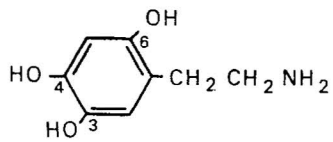
Abnormal transmethylation reactions, involving catecholamines and related compounds, have been postulated as a metabolic basis for schizophrenia. The hydroxylation of the neurotransmitter dopamine (DA, I) to 6-hydroxydopamine (6-OHDA, II) has been advanced as a possible biochemical defect in mental illness (1), and the methylation of 6-OHDA to produce possible psychotoxins chemically related to the known psychotomimetic TMA-2 (IV) has also been proposed (2).

The occurrence of 3,4-dimethoxyphenethylamine (DMPEA, III) in the urine of patients with schizophrenia has been under investigation for many years. Although itself not psychotomimetic, DMPEA could be an indicator of abnormal transmethylation processes. Recently, Braun and Kalbhen (3) have shown, using extremely sensitive detection methods and urine collections for a number of consecutive days, that schizophrenic patients consistently but periodically excrete DMPEA. Enzymes capable of forming bis-O-methylated catecholamine derivatives have been described by Friedhoff et al. (4), and the demethylation of DMPEA has been measured (5). The trimethoxylated compound TMA-2 is also demethylated (6), but the extent of removal of each individual methyl group was not reported.

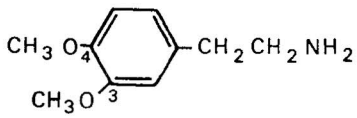
6-OHDA has been the focus of much attention as it appears to produce an irreversible depletion of norepinephrine (NE) and destruction of noradrenergic terminals. Among the one-ring psychotomimetics, the greatest potency is observed with a 2,4,5- ring sub-



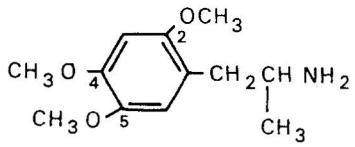
DA (I)



6-OHDA (II)



DMPEA (III)



TMA-2 (IV)

stitution pattern, identical to that of 6-OHDA (II)(2,7). Castagnoli and co-workers (8) have studied the hydrolysis of 2,5-dimethoxy-4-methylphenylisopropylamine (DOM, the 4-methyl analog of IV) and have reported the formation of quinone intermediates after bis-O-demethylation; these products are similar to those observed for 6-OHDA. These considerations have led us to examine the demethylation of both DMPEA and TMA-2 by methods allowing assignment of the source of the methyl group removed.

Materials and Methods

2-$^{14}CH_3O$-labeled TMA-2 was prepared from 3,4-dimethoxyphenol in a four step reaction. The phenol potassium salt in DMSO was coupled with $^{14}CH_3I$, and the resulting anisole converted to 2,4,5-trimethoxybenzaldehyde with N-methylformanilide and $POCl_3$ in a conventional Vilsmeyer reaction. Reaction with nitroethane (acetic acid, ammonium acetate) yielded 1-(2,4,5-trimethoxyphenyl)-2-nitropropene, which was reduced with LAH in ether to yield 2-$^{14}CH_3O$-labeled TMA-2 with a specific activity of 0.75 mCi/mM. The 4-$^{14}CH_3O$- and 5-$^{14}CH_3O$- counterparts were prepared similarly from 2,5-dimethoxyphenol and 2,4-dimethoxyphenol, with specific activities of 0.97 and 1.28 mCi/mM respectively. The preparation of ^{14}C-labeled DMPEA has already been described (5).

Buffalo rats of approximately 300 gm were lightly anesthetized with ether, and the test compound was administered in isotonic solution into a tail vein. Immediately after injection the animal was placed in a 2-liter metabolism cage; air from the cage was

passed through a 400 cc ionization chamber for measurement of ^{14}C and an infra-red detector for the determination of CO_2 (9). One minute average values were submitted to an iterative least squares fitting program on a CDC 7600 computer (10). The resulting curves were expressed in the form $F(t) = A(e^{-r_1 t} - e^{-r_2 t})$, where r_1 and r_2 are the slopes of the two exponential curves of which each data curve is comprised, A is the zero time intercept of back-extrapolation of the slope r_1, and t is the time after injection. The computer program determined the values of A, r_1 and r_2, yielding the function which best fit the observed data. The half times T were obtained by $T = \ln 2/r$. The computer also calculated the integral of the function from zero to 120 minutes, which is the fraction of the injected dose demethylated and expired as $^{14}CO_2$ during this period.

Results and Discussion

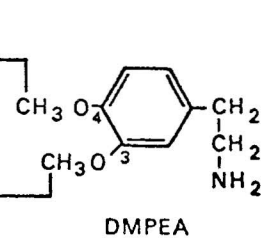
The values obtained from the experiments with the three labeled forms of TMA-2, and from the earlier experiments with the two labeled forms of DMPEA (5), are presented in Table I. The dosage values, the means of the half times T_1 and T_2, and the total percent demethylation for each of the five labeled compounds are shown.

In each set of experiments with a single compound (either DMPEA or TMA-2) the ^{14}C was in a different methoxyl position, so that the differential metabolism of the groups within the same molecule can be compared. Those parameters of a respiration curve which are constant regardless of labeled position probably repre-

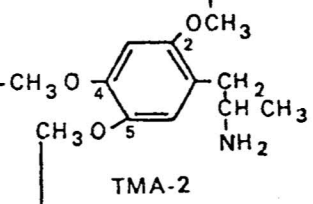
TABLE I

Dosage for each animal and fitting parameters derived for each $^{14}CO_2$ output curve.

Dosage uMol/Kg		A ($\times 10^{-3}$)	T_1 (min)	T_2 (min)	Total % demethylation
47.6		1.12	90.0	5.6	13.5
52.4		2.37	41.0	6.7	11.8
50.3		2.27	51.7	5.0	15.3
	mean	1.92	60.9	5.8	13.5
51.5		0.135	89.9	11.0	1.5
52.7		0.682	36.3	22.0	1.4
87.9		0.180	30.0	8.6	1.8
	mean	0.332	68.7	13.9	1.6
7.1		0.96	46.2	6.9	5.4
13.5		0.90	75.3	2.8	9.7
45.6		1.50	42.8	6.7	7.9
47.7		1.11	64.2	4.4	9.5
	mean	1.12	57.1	5.1	8.1
1.2		3.31	51.3	1.5	23.8
7.1		4.60	40.1	1.3	25.7
8.9		3.45	43.0	1.5	20.6
17.9		2.72	41.0	2.5	15.1
	mean	3.52	43.9	1.7	21.3
1.9		2.14	47.5	5.4	13.0
6.9		2.16	57.8	4.0	16.8
18.6		1.33	74.5	2.7	13.7
29.3		1.65	52.3	5.8	11.2
	mean	1.82	58.1	4.5	13.7



DMPEA



TMA-2

sent factors not directly involved in demethylation, such as transport and oxidation to CO_2. Those parameters which do vary with label position may then be assumed to reflect differences in metabolic activity related to each specific labeled site.

In evaluating expired $^{14}CO_2$, one of the compartments must include the CO_2:HCO_3^- pool. The half times T_1 were nearly the same in all experiments and were assumed to represent the turnover of this pool. The half times T_2, on the other hand, vary widely depending on the position of the label, and will be assumed for purposes of discussion to be directly associated with demethylation.

TMA-2 differs from DMPEA in that it has a third methoxyl group (in the ortho-position of the ring) and an alpha-methyl group on the side chain. A comparison of the rates of demethylation of these two compounds shows that the methyl carbons of the para- and the meta- methoxyl groups are removed some three times faster from TMA-2 than from DMPEA (para, $p < .01$; meta, $p < .025$). Comparing the total percent of demethylation, the para-methoxyl group is about half as extensively removed with DMPEA as with TMA-2 ($p < .05$). However, for the meta-methoxyl group, the total percent demethylation is an order of magnitude greater in TMA-2 than in DMPEA ($p < .001$). It is tempting to associate this sizable increase with the presence of the additional (ortho) methoxyl group. Chemically these two groups (ortho and meta), being located para to one another would, upon bis-O-demethylation, give rise to a hydroquinone or a benzoquinone product. Mechanisms involving such intermediates have been proposed in explanation of the activity of these compounds (2). The lower demethylation rates of DMPEA and the inability to form a para-quinone may explain its lack of psychotomimetic activity.

This implied bis-O-demethylation is consistent with both chemical and biochemical work of others. In a chemical system it has been shown that DOM is bis-O-demethylated to form the p-hydroquinone and the p-quinone, which finally cyclizes to form 5-hydroxy-2,6-dimethylindole (8). Similar processes are reported to occur for 6-OHDA and for the 4-methyl ether analog of 6-OHDA (11).

In biochemical studies with DOM in rabbit liver homogenates demethylation at the 2- and the 5- positions has been measured (8). The total demethylation at the 2- position was about the same for DOM (7-11%) as that observed here for TMA-2 (5.4-9.6%), but the elevated amount of 5-demethylation of TMA-2 (11.2-16.8%) compared to DOM (2.4-6%) may reflect the ability of TMA-2 to form an ortho-quinone as well as a para-quinone. The ortho-quinone cannot occur in DOM, due to the presence of the fixed 4-methyl group. The similarities between the chemistry of DOM and 6-OHDA have led Castagnoli *et al.* to speculate that the pharmacologic basis of action of the two compounds may also be similar (8).

In summary, we have employed a method that allows direct measurement of demethylation of compounds of pharmacologic interest in living animals. The comparison of DMPEA and TMA-2 by this technique supports the concept that methoxylated psychotomimetics may be bis-O-demethylated and cyclized to indolic compounds.

Acknowledgement

This work was supported by the U.S. Energy Research and Development Administration.

References and Footnotes

1. Stein, L. and Wise, C.D., Science 171, 1032-1036 (1971).

2. Shulgin, A.T., Sargent, T. and Naranjo, C., Nature 221, 537-541 (1969).

3. Braun, G. and Kalbhen, D.A., Arch. Psychiat. Nervenkr. 218, 195-210 (1974).

4. Friedhoff, A.J., Schweitzer, J.W., Miller, J. and Van Winkle, E., Experientia 28 517-519 (1972).

5. Sargent. T.W., Israelstam, D.M., Shulgin, A.T., Landaw, S.A. and Finley, N.N., Biochem. Biophys. Res. Comm. 29 126-130 (1967).

6. Mitoma, C., Proc. Soc. Exp. Biol. Med. 134, 1162-1164 (1970).

7. The chemically correct numbering for both compounds is 2,4,5; however, as the discovery of 6-OHDA followed from studies of dopamine with a 3,4-substitution, the dopamine terminology is usually encountered. The different numbering patterns are given in the structural formulae to avoid error.

8. Castagnoli, N., Zweig, J.S. and Weinkam, R., In Advances in Mass Spectrometry (A. Frigerio and N. Castagnoli, eds.). Spectrum Press, New York (in press).

9. Tolbert, B.M., Kirk, M. and Baker, E.M., Am. J. Physiol. 185, 269-274 (1956).

10. Pierson, R.N., Jr., Kusubov, N., Lemmon, R., Landaw, S. and Winchell, H.S., J. Nucl. Med. 15, 118-123 (1974).

11. Ho, B.T., Estevez, V. and Fritchie, G.E., Brain Res. 29, 166-169 (1971).