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Demethylation of C^{14} 2,3,4-Trimethoxyphenylethylamine in Schizophrenics Before and After L-methionine Loading

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Summary: Demethylation was compared in acute and chronic schizophrenics and in non-schizophrenics by the administration of C^{14} labelled 2,3,4-trimethoxyphenylethylamine (TMPEA). The results did not show a significant difference in the urinary levels of the monodemethylated catabolites of TMPEA among any groups of patients. However, there was a significant decrease in demethylation in untreated chronic schizophrenics after ten days of L-methionine orally, whereas the non-schizophrenic group similarly given methionine did not change. This suggests the possibility of a biological weakness in schizophrenia.

Slotta and Muller (1936) reported that 2,3,4-trimethoxyphenylethylamine (TMPEA), an analogue of mescaline, when given orally to schizophrenics, induced an exacerbation of their symptoms. However, when given in the same dose to normal subjects it did not have a hallucinogenic effect. Pollin *et al* (1961) reported that L-methionine (L-me), the main methyl donor in the body (Meister, 1965), can precipitate an acute psychosis in chronic schizophrenics on monoamine oxidase inhibitors (MAOI), followed in some by amelioration of their symptoms. This finding was later confirmed by Brune and Himwich (1962), Alexander *et al* (1963) and Kakimoto *et al* (1967), and extended to patients not receiving MAOI (Antun *et al*, 1971a).

Smythies *et al* (1967) showed that at least three methoxy groups with a 3,4,5 configuration were necessary for hallucinogenic activity of phenylethylamine. The addition of a methoxy group on the 2 and/or 6 position seemed to enhance these properties by rendering the molecule resistant to deamination. Similar configurations were found to be necessary for hallucinogenic activity in amphetamines, the 4; 2,5; 2,4; 2,4,5; and 2,4,6 positions being hallucinogenic (Shulgin *et al*, 1969). These findings suggest that more than one configuration might render the molecule hallucinogenic and that this property might be related to the steric configuration of the methoxy group of the molecule (Antun *et al*, 1971b).

In the light of the transmethylation theory of schizophrenia (Osmond and Smythies, 1952), Slotta and Muller's observations might be explained on the basis of defective demethylation. It is possible that normal subjects can demethylate TMPEA at the *para* position, thus rendering it inactive, whilst schizophrenics cannot; this would be because the 2,3 compound is inactive, the 3,4 compound is very weakly active while the 2,4 compound is very active. This is further supported by the finding that catechol ring demethylation occurs primarily on the *para* position (Axelrod, 1959).

The present study was undertaken to investigate this possibility and test whether under L-me loading a difference in demethylating capacity exists between schizophrenics and non-schizophrenics. We synthesized and administered C^{14}-labelled TMPEA to schizophrenics and non-schizophrenics. We separated and identified its monodemethylated catabolites in the subjects' urine. The dose of C^{14} TMPEA administered was well below dosages reported in the literature for C^{14} radioisotopes (Israelstam *et al*, 1970).

Material and Methods

The monodemethylated metabolites of TMPEA were synthesized according to published methods with minor modifications. These metabolites include 3-hydroxy-2,4-dimethoxy phenylethylamine (Brossi and Teitel, 1969), 4-hydroxy 2,3-dimethoxy phenylethylamine and 2-hydroxy-3,4-dimethoxy phenylethylamine. The latter two were prepared by lithium aluminium hydride reduction of the corresponding nitrostyrenes, which were prepared from the corresponding benzaldehydes and phenols (Duff, 1941; Worral, 1941). C^{14} TMPEA was synthesized using the methods described by Kubota and Masui (1965). Rf values of the different metabolites of TMPEA are shown in the legend to Fig 1.

Seven groups of patients were selected from a hospital population who were standardized for diet (Table 1). Diagnosis of schizophrenia was confirmed by two independent psychiatrists, using the ICD 9

TABLE I

The different patient groups used in the clinical studies

Group	Category	Male/Female	Age range
I	Chronic schizophrenics not receiving drugs	5/0	29–75
Ia	4 chronic schizophrenics from group I receiving L-methionine	4/0	29–72
II	Chronic schizophrenics who received chemotherapy	5/5	21–58
III	Manic depressives (as control group)	5/5	19–39
IIIa	4 male manic depressives from group III receiving L-methionine	4/0	23–35
IV	Acute schizophrenics before chemotherapy	5/4	51–71
IVa	4 male acute schizophrenics from group IV after chemotherapy	4/0	51–65

classification. The project was explained to the patients and written consent was obtained.

Fifty milligrams of C^{14} TMPEA, equivalent to 3.5×10^6 cpm, in 1 ml of saline were injected into each patient intramuscularly. A 24-hour urine sample was then collected indivudually in 6N HCl. Strict supervision was observed over the urine collection. Urinary pigments were removed with florisil, and the filtrate boiled for 30 minutes. After cooling, the urine was made alkaline (pH 8.5), extracted with n-butanol and concentrated. The concentrate was chromatographed on Whatman No 1 paper using n-butanol:acetic acid:water (4:1:5 v/v) as eluent. The metabolites were identified by colour reaction with Gibb's reagent. The chromatography paper was divided into 1×2 cm strips starting from the origin to the solvent front. The strips were cut and placed in special plastic vials containing scintillation solution (POP, POPOP, toluene). The number of counts of each metabolite was deduced from the histogram drawn for each sample (Fig 1). The average total urinary recovery of the amines amounted to 45 per cent of the total counts administered.

An oral daily dose of 20 grams of L-me was given to groups Ia and IIIa (Table I) for 10 consecutive days. C^{14} TMPEA was administered on the 11th day, the urine collected over 24 hours and analysed as described above. Patients of group IVa were treated with standard antipsychotic drugs consisting of phenothiazines or butyrophenones. Ten weeks later C^{14} TMPEA was administered intramuscularly, urine was collected over 24 hours and analysed as described above.

Results

The non paired t-test showed no statistically significant difference ($P > 0.05$) in demethylation at the 2,3, and 4-methoxy positions between the following groups:

Group III vs (I+II)
Group III vs IV
Group IV vs (I+II)

The student paired t-test showed no statistically significant difference ($P > 0.05$) in demethylation of the 2,3 and 4-methoxy positions between the following groups:

Group III vs IIIa
Group IV vs IVa

In group Ia, however, the results of the student

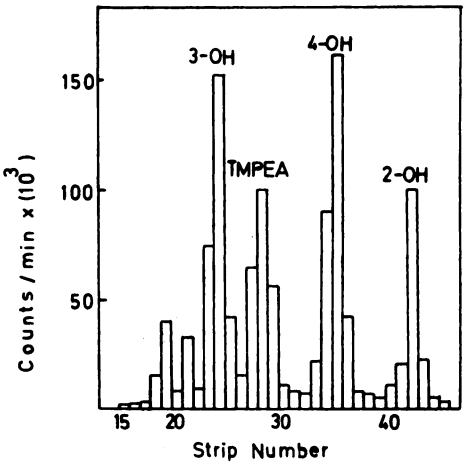

FIG 1.—A histogram of the average number of counts/min for each 1×2 cm paper strip cut out of the chromatography paper sheet, spotted with human urine extract, starting from the 15th cm band from the origin.

3-OH 3-hydroxy-2,4-dimethoxy phenylethylamine Rf 0.48
4-OH 4-hydroxy-2,3-dimethoxy phenylethylamine Rf 0.68
2-OH 2-hydroxy-3,4-dimethoxy phenylethylamine Rf 0.9
TMPEA 2,3,4-trimethoxy phenylethylamine Rf 0.56

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TABLE II

The number of counts/minute ± SEM. Relative percent values of each monodemethylated metabolite and TMPEA calculated from their sum total

Group	3-hydroxy	TMPEA	4-hydroxy	2-hydroxy
I	141 558 ± 41 527 27.8%	94 093 ± 40 650 18.5%	180 725 ± 64 380 35.6%	92 549 ± 30 728 18.2%
II	248 298 ± 33 656 30.4%	172 587 ± 29 788 21.1%	273 401 ± 42 543 33.4%	123 590 ± 18 222 15.2%
III	151 052 ± 17 273 19.6%	216 948 ± 25 576 28.2%	270 863 ± 29 198 35.2%	129 539 ± 20 562 16.9%
IV	75 694 ± 13 903 17.0%	129 998 ± 29 587 29.0%	162 650 ± 29 213 36.6%	76 099 ± 14 697 17.3%
Ia	55 579 ± 7 598 18.0%	103 916 ± 8 382 33.7%	57 466 ± 10 463 19.2%	89 543 ± 9 875 29.3%
IIIa	217 779 ± 20 698 29.2%	185 992 ± 25 455 20.2%	190 484 ± 33 940 38.8%	88 531 ± 10 560 11.8%
IVa	93 841 ± 19 391 16.5%	150 235 ± 35 509 26.5%	219 901 ± 35 334 38.9%	102 030 ± 24 143 18.1%

paired and non-paired t-tests compared with groups I and IIIa respectively showed a statistically significant ($P < 0.05$) reduction in demethylation at the 3 and 4-methoxy positions. The percentage reduction in demethylation on each was as follows:

(9.8 per cent 3-position, (16.4 per cent) 4-position in groups Ia vs I;

(11.2 per cent) 3-position, (19.6 per cent) 4-position in groups Ia vs IIIa.

There was no significant difference in demethylation at the 2-methoxy positions in the above groups (see Table II).

Discussion

The chronic schizophrenic group after L-me showed decreased demethylation compared to before L-me. This decrease was also significant when the chronic schizophrenic group after L-me was compared with the control group after L-me (groups Ia vs IIIa). We were unable to detect such difference in demethylation between the chronic and/or acute schizophrenics and non-schizophrenics. If the clinical syndrome of schizophrenia is caused genetically by a deficient enzymatic system for demethylation leading to the accumulation of endogenous hallucinogens in the brain, the above findings suggest that perhaps this is due to weak demethylation, which shows itself under biochemical stress, i.e., augmentation of the methylation pool by L-me feeding. The significance of this finding awaits future investigation in a larger group of patients and testing whether such difference correlates with the clinical reaction of some schizophrenics to L-me or is present without L-me loading.

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