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INDOLETHYLAMINE-N-METHYLTRANSFERASE IN SERUM SAMPLES OF SCHIZOPHRENICS AND NORMAL CONTROLS

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SUMMARY

The incidence of N-methyltransferase in blood samples from eleven normal controls, seven acute schizophrenics and eighteen chronic schizophrenics with acute episodes was investigated. The presence of the enzyme was determined by a simple, but highly sensitive, method using two-dimensional thin-layer chromatography and o-phthaladehyde spray reagent. Serotonin (5-HT) and 5-methoxytryptamine (5-OMeT) were used as substrates.

The normal samples did not show any bufotenin spots when 5-HT was used as the substrate or 5-methoxy-N:N-dimethyltryptamine (5-OMeDMT) spots with 5-OMeT as the substrate. Of the seven samples from the acute schizophrenics, six showed spots isographic with bufotenin or 5-OMeDMT, and thirteen of the eighteen samples from the chronic schizophrenics were positive for bufotenin or 5-OMeDMT.

The presence of the three psychotogenic N,N-dimethylated tryptamines, N,N-dimethyltryptamine (DMT), 5-methoxy-N,N-dimethyltryptamine (5-OMeDMT), 5-hydroxy-N,N-dimethyltrypamine (bufotenin) in urine and blood samples of schizophrenics has been demonstrated in a series of studies reported from our laboratories (1). Selective methods of separation, sensitive methods of identification by both two-dimensional thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) have been used for the identification of these compounds.

The occurrence of a nonspecific enzyme capable of N-methylating indolethylamines was first reported by Axelrod (2) who showed its presence in rabbit lung. More recently Mandell and Morgan demonstrated the

presence of a relatively specific indolethylamine-N-methyltransferase in chick (3), sheep and human brain (4).

The method most commonly employed in such studies uses serotonin (5-HT) as a substrate and [methyl-^{14}C]S-adenosylmethionine (SAM) as a methyl donor. The main advantage of this method is its high sensitivity; even low concentrations of the N-methylated product can be determined by a liquid scintillation counter. However, the unequivocal identification of the product as the real N-methylated metabolite is a prerequisite in such identification.

In such cases adequate separation procedures, such as TLC and paper chromatography, are used to purify the product before measuring the specific activity of the methylated product. However, this method could be expensive for screening a large number of biological samples. Any alternative method equally sensitive and selective in separating the substrate from the product should prove adequate for studying the N-methyltransferase levels in biological samples.

We previously reported a very sensitive method using o-ophthalaldehyde (OPT) for the identification and quantitation of several 5-hydroxytryptamine derivatives. The sensitivity of the method is in the range of .003 µg for 5-HT, bufotenin, 5-methoxytryptamine (5-OMeT), and 5-OMeDMT (5). In this study we showed that the N-dimethylated compounds were well separated from corresponding primary amines.

The occurrence of N-methyltransferase in normal human brain (4) prompted us to investigate its incidence in blood samples of schizophrenic patients and normal controls, and to study the qualitative and quantitative differences between the two groups with this highly sensitive method by using both 5-HT and 5-OMeT as substrates. The results of the investigation are the subject of this communication.

Materials and Methods

Blood samples were collected from eleven normal controls, seven acute schizophrenics and eighteen chronic schizophrenics with acute episodes. The

serum was separated by centrifugation, frozen and then lyophilized. Samples were stored at -30°C until used (from 1 to 12 weeks). 5-HT or 5-OMeT was used as a substrate.

The commercial sample of 5-OMeT was not chromatographically pure and was therefore recrystallized from ethyl acetate to obtain a chromatographically pure material. SAM (S-adenosyl-L-methionine chloride) was obtained from the Sigma Chemical Company, St. Louis. Solutions of 5-HT (serotonin creatinine sulphate), SAM and 5-OMeT were prepared by dissolving 5-HT and SAM in water and 5-OMeT in 0.05 N HCl in amounts to give a concentration of each compound of 1 mg/ml.

Two hundred mg of lyophilized serum sample were dissolved in 1 ml of phosphate buffer (0.2 M, pH 7.1); 100 µl of the 5-HT and SAM solutions (100 µg of each compound) were added, and the mixture was incubated at 37°C for 4 hours. A blank consisting of 200 mg of serum only in 1 ml of buffer was incubated simultaneously. At the end of 4 hours the blank and the test solutions were treated with a few drops of 60% perchloric acid and centrifuged. The precipitate was washed with 1 ml of distilled water and the supernatant and washings were pooled. The pH was adjusted to 10.0 with ammonia and the solution was extracted with 10 ml of ethyl acetate. The ethyl acetate, after being dried with Na_2SO_4, was removed by evaporation under vacuum and the residue was redissolved in 100 µl of ethyl acetate. Ten-µl aliquots of the solution were spotted on a two-dimensional TLC plate (silica gel G) with 5-HT and bufotenin as reference spots. The blank sample was also spotted on either side of the TLC plate alongside the standard samples.

In another series of experiments 100 µl of a solution of 5-OMeT replaced 5-HT as substrate. The procedure was the same as with 5-HT except for this substitution and the adjustment of the pH to above 10 with aqueous sodium hydroxide (2N) after treatment with perchloric acid at the end of the 4-hour incubation period. Chloroform was also used for extraction. When 5-OMeT was the substrate, 5-OMeT and 5-OMeDMT were used as reference standards.

In control experiments, two mixtures, one containing 5-HT and bufotenin and the other 5-OMeT and 5-OMeDMT (100 µg:1 µg/100 µl), were prepared and 10-µl aliquots were spotted on two-dimensional TLC using solvent systems, chloroform:methanol:water 12:7:1 (I) and isopropyl alcohol:ammonia:water 85:5:15 (II). The plates were sprayed with OPT reagent and fluorescence observed under UV after heating at 100°C .

For purposes of correct identification, the spots corresponding to bufotenin or to 5-OMeDMT were always scraped, eluted with 6 N HCl and F_{max} was determined at activation 360 nm. If F_{max} was found at 480-490 nm, the fluorescence reading was obtained along with the reading for the reference spot. As an additional test an aliquot of the concentrate was heated with 50 µl of a 0.05% solution of OPT in methanol and 100 µl of concentrated HCl for 30 minutes and the reaction mixture spotted on TLC. This modified OPT method gives two spots for each compound and here again the two spots of the substrate and two spots of the product are well separated (see reference 5).

Results

The quantity of serum used was equivalent to 2 ml and the aliquot spotted on TLC was equivalent to 0.2 ml. At this level there is no evidence of detectable endogenous components which would interfere with the identification of the enzymatic conversion products. The blanks did not show any fluorescent spots except in rare instances when a faint spot for 5-HT was observed.

The R_F values of 5-HT and bufotenin and of 5-OMeT and 5-OMeDMT are shown in Table 1. Because of the high sensitivity of the OPT reagent, even a 1% conversion of the substrate to the product by the enzyme became detectable.

In the control experiments with mixtures of 5-HT and bufotenin and of 5-OMeT and 5-OMeDMT a distinct separation of the substrate and the product with no tailing, overlapping or background fluorescence was clearly evident after OPT spray. With 5-OMeT as a substrate the extraction efficiency was increased because of more favorable partition, greater stability and greater flexibility in pH adjustment.

TABLE 1

R_F values of 5-HT — bufotenin and 5-OMeT — 5-OMeDMT*

Solvent systems	5-HT — bufotenin	5-OMeT — 5-OMeDMT
$CHCl_3$:CH_3OH:H_2O		
12:7:1	0.35 — 0.78	0.67 — 0.93
24:7:1	0.15 — 0.39	0.41 — 0.78
IPA:NH_4OH:H_2O		
85:5:15	0.28 — 0.44	0.36 — 0.52
85:10:15	0.43 — 0.62	0.54 — 0.71

* Solvent front, 6 inches

In the test samples, none of the serum samples from the eleven normals showed any bufotenin spots when 5-HT was used as the substrate or 5-OMeDMT spots with 5-OMeT as the substrate. Six of the seven samples from the acute schizophrenics showed spots isographic with bufotenin or 5-OMeDMT. Of the eighteen samples from the chronic schizophrenics with acute exacerbations, thirteen showed positive results for the enzyme activity.

TABLE 2

The products of N-dimethylation by serum samples from acute and chronic schizophrenics, and their fluorescence readings after o-phthalaldehyde (OPT) spray.

Sera	Substrate	Product	Fluorescence	Blank
A[1]	5-HT	Bufotenin	0.385	0.077
A	5-HT	Bufotenin	1.38	0.058
A	5-OMeT	5-OMeDMT	0.294	0.043
C[2]	5-OMeT	5-OMeDMT	0.465	0.065
C	5-OMeT	5-OMeDMT	2.49	0.065
C	5-OMeT	5-OMeDMT	0.885	0.066
C	5-OMeT	5-OMeDMT	1.035	0.034
C	5-OMeT	5-OMeDMT	2.205	0.013
C	5-OMeT	5-OMeDMT	0.261	0.050
C	5-OMeT	5-OMeDMT	0.43	0.043

[1]A, acute schizophrenic; [2]C, chronic schizophrenic

Examples typical of our results are given in Table 2. The fluorescence readings obtained with the product spots are also shown.

In a few instances the serum samples were tested for the N-methyl transferase activity within 2 days after collection and the same samples retested after being stored for 6 months. There was no loss of enzyme activity on storage at -30°C after lyophilization.

Discussion

The method reported here for the identification of N-methyltransferase in human blood is simple, but highly sensitive, and is a good substitute for the conventional method usually employed in such studies which uses SAM with ^{14}C-methyl donor.

While this communication was in preparation a paper by Heller (6) on N-methylating enzyme in the blood of schizophrenics was published in which the author used 5-HT (1 µM) as substrate and 0.2 ml of plasma for the enzyme. The author claimed to have identified the product, N-methyl 5-HT, by thin-layer chromatography and OPT spray.

In our opinion based on our experience with the solvent systems used in Heller's (6) study, the possible separation of 5-HT and N-methyl 5-HT would be masked by the relatively large amount of substrate as compared to the amount of enzyme. The quantity of plasma used (0.2 ml) is too small to cause any appreciable methylation, and in the presence of such a large excess of 5-HT (176 µg), N-methyl 5-HT could not have been seen as a separate spot. It therefore seems unlikely that the presence of N-methyltransferase could have been demonstrated by the method reported.

In our present method both substrates, 5-HT and 5-OMeT, are well separated from the products of N-dimethylation, and mixtures containing the substrate and product in a 100:1 ratio have been separated. We have shown by this simple technique that the N-methyltransferase is detectable in acute and chronic schizophrenics and not in normals.

Although we have not done a large enough number of samples to permit a

statistical evaluation of our results, we have reported the method so that it could be used for large scale screening. The results here are qualitative showing only that N-methyltransferase activity is not detectable in normal controls, but is present at detectable levels in both chronic and acute schizophrenics. However, this method is capable of quantitative evaluation by the estimation of the products, bufotenin and 5-OMeDMT, by spectrofluorometry after OPT spray (see Table 2).

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