4-METHOXYPHENYLETHYLAMINE AND 3,4-DIMETHOXYPHENYLETHYLAMINE IN HUMAN URINE

N.P. Sen and P.L. McGeer
Kinsmen Laboratory of Neurological Research,
The University of British Columbia, Vancouver, Canada

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Friedhoff and Van Winkle (1962) reported the presence of 3,4-dimethoxyphenylethylamine in the urines of acute schizophrenic patients but not in those of controls. Takesada et al. (1963) found this metabolite in the urines of many controls as well as schizophrenics. Identification in both cases was based upon paper chromatography.

Subsequently, Friedhoff and Van Winkle (1963) infused dopamine hydrochloride-7-^{3}H into schizophrenic patients, recovered labelled 3,4-dimethoxyphenylacetic acid, and recrystallized it to constant specific activity. They also showed that liver tissue taken from a schizophrenic patient could methylate dopamine in the 4- as well as the 3- position in vitro.

These findings prove that methylation of certain 4-hydroxybenzene derivatives can take place in humans. Furthermore, they suggest the possibility that this may occur more readily in schizophrenics.

This paper is to report evidence concerning the presence of a new compound, 4-methoxyphenylethylamine, in human urine. The evidence was obtained in the course of paper and gas chromatographic experiments which also gave further confirmation of the presence of 3,4-dimethoxyphenylethylamine in human urine.

Extraction Procedure: Approximately 400 ml (equivalent to 350-400 mg of creatinine) of each 24-hour urine specimen was acidified to pH 1 with HCl and extracted three times with an equal volume of ether. The aqueous residue was treated with 1 gm of ethylenediaminetetraacetic acid, adjusted to pH 9 with 3N NaOH, and the mixture shaken for five minutes with 800 ml of chloroform (glass-distilled). A further 10 ml of 3N NaOH

was added and the mixture shaken vigorously for seven minutes. The aqueous layer was extracted with two additional 600 ml portions of chloroform. The combined chloroform extracts were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to a small volume.

Paper Chromatography: An extract equivalent to about 100 ml of urine was analyzed by descending paper chromatography (Whatman No. 40 filter paper) at 22°C, using isopropanol-ammonium hydroxide-water (8:1:1) in the first dimension, followed by n-butanol-acetic acid-water (4:1:1) in the second dimension. The chromatograms were developed with ninhydrin.

R_f data for 4-methoxy- and 3,4-dimethoxyphenylethylamine are given in Table 1. Ninhydrin-positive spots chromatographically identical with authentic 4-methoxyphenylethylamine and 3,4-dimethoxyphenylethylamine were present in many chromatograms. Addition of the appropriate standards to urine extracts always enhanced the spots.

Further proof of the identity of these spots was provided in a number of cases by gas chromatography.

Table 1.
Chromatographic properties of
4-methoxy- and 3,4-dimethoxyphenylethylamine

Amine	R_f values in solvent systems	
	Isopropanol-conc. NH_4OH-water (8:1:1)	n-Butanol acetic acid-water (4:1:1)
4-Methoxyphenylethylamine	0.80	0.63
3,4-Dimethoxyphenylethylamine	0.74	0.58

Preparation of Samples for Gas Chromatography: Part A, Isolation by Paper Chromatography. Chromatograms of chloroform extracts of urine with and without added 4-methoxyphenylethylamine and 3,4-dimethoxy-

phenylethylamine (20 micrograms each) were run as previously described. Areas corresponding to 4-methoxyphenylethylamine and 3,4-dimethoxyphenylethylamine were cut out and eluted with a few mls of 0.01 N HCl. Exact spot location was determined in each case by comparison with a duplicate chromatogram sprayed with ninhydrin; correspondence between pairs of chromatograms was confirmed through ultraviolet spots (2570 Å) which appeared on all unsprayed chromatograms.

Derivatives suitable for gas chromatography were then prepared from these eluants. In each case the whole eluant was used to prepare one derivative.

Part B. Preparation of Acetyl Derivatives. The eluant was made alkaline and then extracted three times with 30 ml portions of chloroform. The chloroform layer was dried over anhydrous Na_2SO_4 and concentrated to dryness. About 0.5 ml acetic anhydride was added and the mixture was gently refluxed for three minutes. After cooling, the excess acetic anhydride was destroyed by the addition of 6 ml of 3N NaOH. Finally, the acetyl derivative of the amine was extracted with glass-distilled ether, the ether layer dried over anhydrous Na_2SO_4, and concentrated to 0.1 ml. This solution was used for gas chromatography.

Part C. Preparation of Benzoyl Derivatives. Two mls of 10N NaOH and 0.5 ml of benzoyl chloride were added to the eluant. The mixture was shaken for one hour, after which the pH was adjusted to 11.8, and the benzoyl derivative extracted with glass-distilled ether. The ether layer was dried over anhydrous Na_2SO_4 and concentrated to 0.1 ml. This solution was used for gas chromatography.

Conditions for Gas Chromatography: A Perkin Elmer Model 800 gas chromatograph equipped with dual columns and a differential flame ionization detector was used. The columns were 1/8" stainless steel tubes of various lengths. Details of the stationary phases and other conditions used, along with the retention times of different derivatives, are given in Table 2.

Table 2.
Retention times in minutes of N-acetyl- and N-benzoyl derivatives of amines

	SE-30[a]		NGS[a]	APL[a]
	240°C	260°C	210°C	220°C
Acetyl 4-methoxyphenyl-ethylamine	*	*	3.4	2.8
Acetyl 3,4-dimethoxy-phenylethylamine	*	*	7.7	5.2
Benzoyl 4-methoxyphenyl-ethylamine	3.8	*	**	**
Benzoyl 3,4-dimethoxy-phenylethylamine	**	2.9	**	**

[a] Column packing:
6% coating of SE-30 on 60-80 mesh chromosorb W; 6 ft. in length. Flow of N_2, 40 ml per minute.
2% neopentylglycolsuccinate (NGS) on 80-90 mesh Anakrom ABS; 3 ft. in length. Flow of N_2, 62 ml per minute.
5% Apiezon L (APL) on 80-90 mesh Anakrom A; 4 ft. in length. Flow of N_2, 40 ml per minute.

* Derivatives passed through the column too quickly for accurate analysis.

** Derivatives passed through the column too slowly to be suitable for analysis.

Two to 4 µl aliquots of unknown solutions were injected into the column with and without added standards. A positive result was recorded only in cases where the unknown and the appropriate standard appeared on the recording chart as a single peak.

Figure 1, for example, demonstrates gas chromatographic evidence for the presence of the acetyl derivative of 4-methoxyphenylethylamine in one urine extract. Curve A is for the acetyl derivative of authentic 4-methoxyphenylethylamine, curve B for the acetyl derivative of material isolated from urine and prepared as described above, and curve C for the urinary derivative plus added standard N-acetyl-4-methoxyphenylethylamine. The dotted line represents a blank. Identical blanks were found if the acetylation was carried out on eluants of 4-methoxyphenylethylamine areas from chromatograms of urines not containing the amine, or from adjacent areas of chromatograms of urinesin which the amine was present.

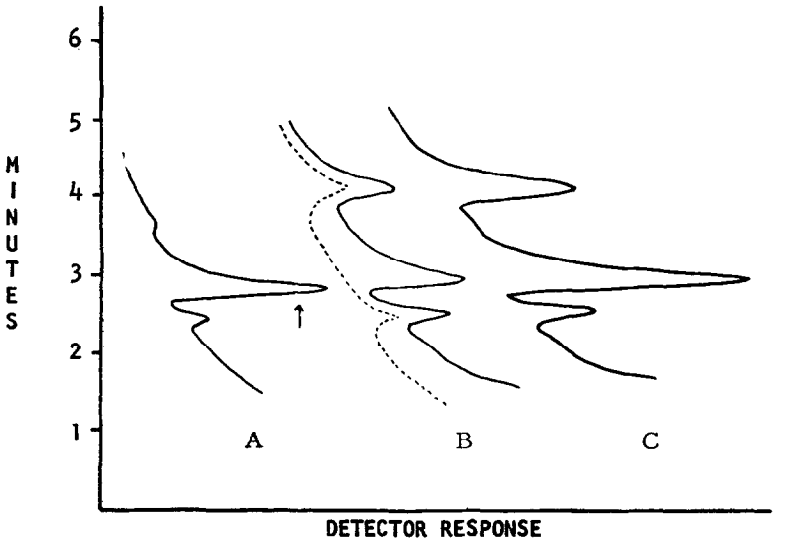

Figure 1. Gas chromatography of the acetyl derivative of 4-methoxyphenylethylamine (the peak marked with an arrow). Conditions: Column 4 ft. x 1/8" I.D. stainless steel tube, 5% Apiezon L on 80-90 mesh Anakrom A; temperature 220°C, nitrogen flow 40 ml per minute. For description see text.

Our evidence for the existence of 4-methoxyphenylethylamine and 3,4-dimethoxyphenylethylamine in urine consists therefore of the following;

(a) Paper chromatographic identity of the materials found in urine extracts with authentic standards in two solvent systems,

(b) Gas chromatographic identity of the acetyl derivatives of the materials isolated by two-dimensional paper chromatography with the acetyl derivatives of authentic standards in two different columns, and

(c) Gas chromatographic identity of the benzoyl derivatives of the isolated materials with the benzoyl derivatives of authentic standards in one column.

Results and Discussion. Of fifteen urine samples from acute schizophrenic patients examined by paper chromatography alone, seven contained a compound indistinguishable from 4-methoxyphenylethylamine, and one contained compounds indistinguishable from both 4-methoxyphenylethylamine and 3,4-dimethoxyphenylethylamine.

Of seven urine samples from further acute schizophrenic patients examined by both paper and gas chromatography, three contained compounds indistinguishable from the two amines, and one contained a compound indistinguishable from 3,4-dimethoxyphenylethylamine only.

A normal urine sample showing no evidence of either 4-methoxy- or 3,4-dimethoxyphenylethylamine was mixed with 10 µgs of each amine and then submitted to the extraction and chromatographic procedures described above. From a comparison of the areas of the gas chromatographic peaks it was estimated that the positive urines examined contained about 5-20 µgs of each amine in a 24-hour specimen. Considerably larger quantities of 3,4-dimethoxyphenylethylamine would be implied from the studies of Friedhoff and Van Winkle (1962) and Takesada et al. (1963).

Further work on the metabolic origins of these compounds, and their significance, if any, to pathological conditions such as schizophrenia, is in progress and will be reported in detail elsewhere.

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References

Friedhoff, A.J., and Van Winkle, E. Nature, 194, 897 (1962).
Friedhoff, A.J., and Van Winkle, E. Nature, 199, 1271 (1963).
Takesada, M., Kakimoto, Y., Sano, I., and Kaneko, A., Nature, 199, 203 (1963).