Chapter 2

Psychotogenic N,N-Dimethylated Indole Amines and Behavior in Schizophrenic Patients

H. Tanimukai, M.D., DMSc., R. Ginther, B.S., J. Spaide, M.S., J. R. Bueno, M.D., and H. E. Himwich, M.D.

INTRODUCTION

The presence of norepinephrine, dopamine, and serotonin in the brain of mammals (including man) has been confirmed repeatedly, and the possible physiological role of these amines in the cerebral functions has been discussed. Many neuropsychotropic drugs used in the treatment of mental patients are known to affect the cerebral level of these primary amines. In contrast, N,N-dimethylated indole amines (in the right column in Fig. 1) have evoked psychotomimetic effects in human beings [1–4] or caused behavioral disturbances in experimental animals [5]. Axelrod [6, 7] has demonstrated by *in vitro* studies that such N,N-dimethylated indole amines could be formed in mammalian tissues from the naturally occurring primary indole amines, such as tryptamine or serotonin. In this reaction, the tryptamine moiety of such N,N-dimethylated indole amines is originally derived from the essential amino acid, tryptophan (Fig. 1), and the methyl groups can be supplied by a methyl donor such as methionine or betaine.

Pollin, Cardon, and Kety [8] have reported that in schizophrenic patients given a monoamine oxidase (MAO) inhibitor, loading with methionine or tryptophan, or both, exacerbated the mental symptoms in some patients. This interesting finding has been confirmed by investigators from different laboratories [9–14]. In 1962, Brune and Himwich [9] suggested that the formation of N,N-dimethylated indole amines may be facilitated in the body under such loading conditions and these tertiary indole amines may mediate the aggravation of the mental symptoms of schizophrenic patients in the presence of a MAO inhibitor. In order to check this hypothetical suggestion, it is necessary to determine whether or not such tertiary indole amines are present in the body fluids of schizophrenic patients, at least at the time when the patients exhibit active psychotic symptoms.

Some authors have reported the presence of a bufotenin-like compound in the urine of schizophrenic patients [15–17] or of normal subjects [18–20]. The detection methods used by these authors, however,

This investigation was supported in part by Public Health Service International Postdoctoral Fellowships No. 1FO5-TW-949-01 and No. 2FO5-TW-949-02.

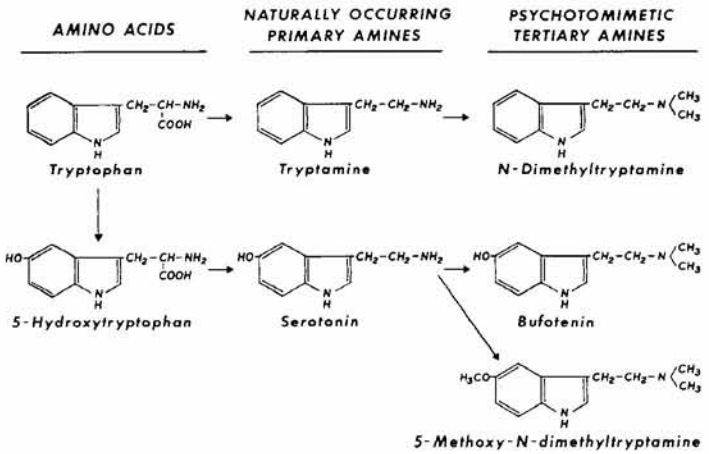

Fig. 1. Chemical structure and possible metabolic pathway of some indole compounds.

are open to criticism [21, 22] so that the reliability of their findings is doubtful. All of these investigators except Gross and Franzen [20] used acetone as a desalting agent or as a solvent in their procedures for purification of urinary indole amines. In the presence of acetone, naturally occurring primary amines are readily transformed into artifactual substances, and some of these unfortunately behave very much like bufotenin on paper chromatograms [21]. The chemical method used by Gross and Franzen [20] was also criticized by Siegel [22]. In contrast, other investigators have failed to find bufotenin or other N,N-dimethylated indole amines in the urine of either schizophrenic patients [22–28] or nonschizophrenic individuals [22, 23, 25, 26, 28]. For the most part, their methods were not sensitive enough to detect small amounts of N,N-dimethylated indole amines excreted in the urine [21], and this lack of sensitive detection methods may be one reason for the confused state of the bufotenin problem.

Recently, one of the present authors [21] developed modifications of paper and thin-layer chromatographic methods which are more sensitive and reproducible than those previously employed for the detection of very small amounts of N-methylated indole amines in the urine. Using these modifications and also the recently developed gas–liquid chromatographic method of Capella and Horning, we examined the urine of four schizophrenic patients under experimental conditions [29].

RESEARCH DESIGN

Four chronic schizophrenic patients with active symptoms, in age from 40 to 58 years, were studied while on a controlled diet which excluded all preformed catechol and indole amines. Psychoactive drugs were withheld from the patients for 4–6 weeks prior to the initiation of

the study and throughout the experimental period. After a seven-day control period, the patients were given, at different times, L-cysteine or tranylcypromine (Parnate), a MAO inhibitor, as well as tranylcypromine plus L-cysteine according to the protocol (see Fig. 2). Clinical evaluations of the mental and behavioral symptoms of these patients were made by a team of physicians during weekly interviews. In addition, daily ward rounds were made and the records of the nursing staff recorded. Twenty-four-hour collections of urine were made and the daily specimens kept at −20°C until analyzed.

METHODS

Detection of indole amines was made in a systematic way (Fig. 3). As details of these methods are published elsewhere [21, 30], only a brief description is given below. One-fourth of a 24-hr urine volume was used for determination. After this volume was concentrated down, the indole amine fractions were purified by separation from other urinary constituents by means of an ion-exchange resin.

By this purification method, indole amines excreted in the free and conjugated forms were obtained separately, and each fraction was then examined separately. High purification of amine fractions at this stage made it possible to apply the following chromatographic methods to large amounts of sample, which helped to increase the sensitivity of the detection. Indole amines were identified by three chromatographic methods, namely, paper, thin-layer, and gas–liquid, as well as by combinations of these methods. The solvent systems used for two-dimensional paper and two-dimensional thin-layer chromatography are shown in Figs. 4 and 5, respectively, in the section "Results." Indole spots were visualized by spraying with 1% p-dimethylaminocinnamaldehyde reagent. One microgram of bufotenin added to 1000 ml of urine was consistently detected by the thin-layer method used in the present study. For gas–liquid chromatographic analysis, an F & M Model 400 gas chromatograph equipped with a hydrogen-flame ionization detector was em-

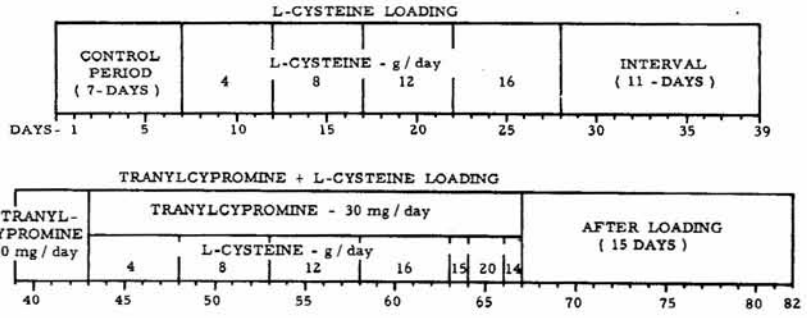

Fig. 2. Experimental schedule for loading of dietary supplements and tranylcypromine, a monoamine oxidase inhibitor.

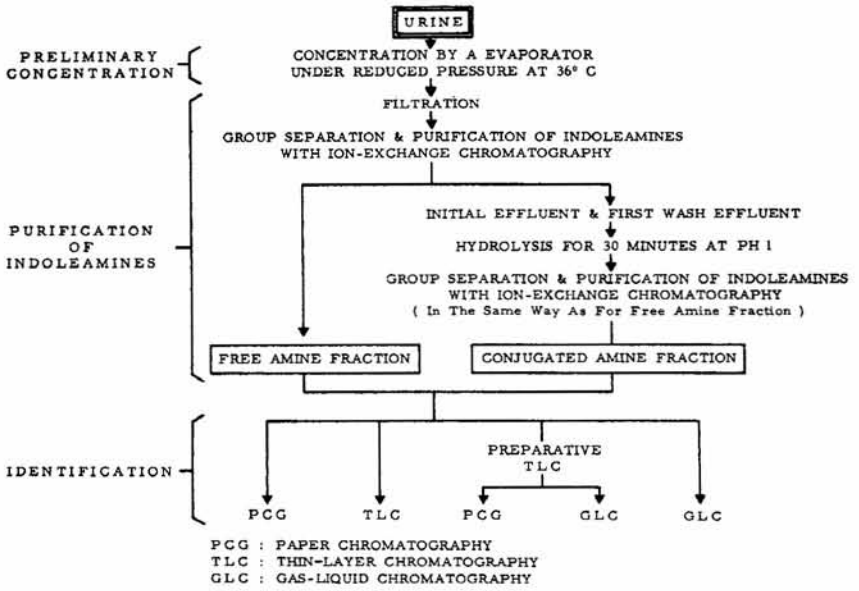

Fig. 3. Systematic representation of methods used for detecting urinary indole amines.

ployed. The column used was a 6 ft × $^1/_4$ in. glass U-tube packed with either (1) 10% F-60 or (2) a mixture of 7% F-60 and 1% EGSP-Z coated on Gas Chrom Q (silanized Gas Chrom P). Temperature-programmed separation was carried out from 150 to 230°C (temperature rise of 2°C/min); the carrier gas was helium or nitrogen. The sample was dissolved in dimethylformamide and then treated with hexamethyldisilazane and acetone successively, according to Capella and Horning [31]. Methylene-unit values were measured by interpolation between retention times for pairs of even-numbered straight-chain saturated hydrocarbons ranging from C_{16} to C_{22}.

RESULTS

A diagram of the paper chromatographic pattern of indole compounds prepared by our method from the urine of schizophrenic patients is shown in Fig. 4. Tryptamine, serotonin, and tryptophan were always found on the chromatograms (Fig. 4). In the absence of MAO blockade, no bufotenin spot was observed by the paper method, while in the presence of a MAO inhibitor both with and without cysteine, we found a blue bufotenin spot in both the free and conjugated amine fractions in a few urine samples from two out of the four patients.

Thin-layer chromatography was much more sensitive than the paper method, and we observed more indole spots on thin-layer chromatograms than on the paper. Tryptamine, serotonin, tryptophan, and two unidentified

spots (Nos. 4 and 5 in Fig. 5) were always noted, regardless of the medication employed. In the absence of a MAO inhibitor, a very faint spot of bufotenin was found (mainly in conjugated forms) in few urine samples. When tranylcypromine was given to the patients, bufotenin was excreted in relatively larger amounts in the urine, and bufotenin was detected consistently in all four patients examined. At that time both free and conjugated forms of bufotenin were disclosed in practically all examinations. The identification of bufotenin was based on the R_f values and coloration of the suspected spots on two-dimensional paper and two-dimensional thin-layer chromatograms; these results were completely identical with those obtained with the authentic compound. Further proof of the identification was obtained by gas–liquid chromatographic analysis. As outlined by Capella and Horning [31], all substituted hydroxyl groups in the sample were converted to trimethylsilyl ether groups before the sample injection. Moreover, the samples were examined both before and after the acetone reaction. Unlike paper chromatography, acetone treatment of the sample did not interfere with the gas–liquid chromatographic identification of N,N-dimethylated indole amines because the acetone condensation products of primary amines were distinctly separated from the tertiary amines on gas–liquid chromatograms. A bufotenin peak showing the same methylene-unit value as that of the authentic compound was clearly detected on the chromatograms (Fig. 6). Trypta-

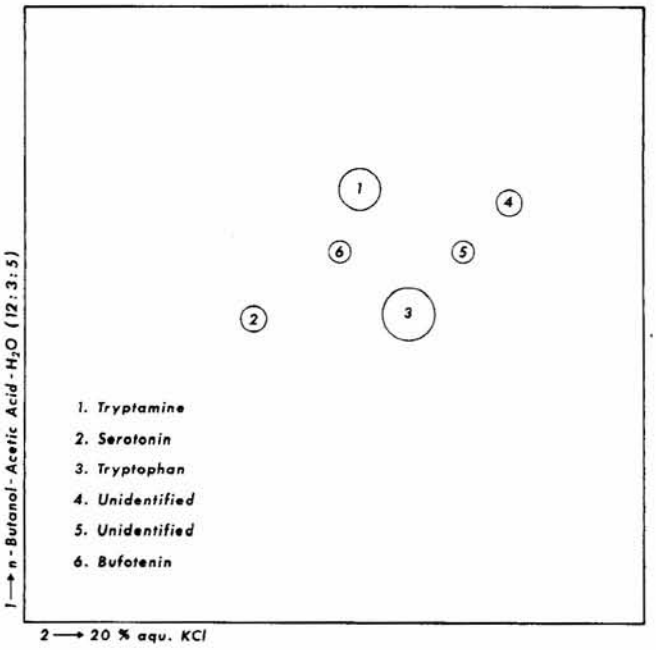

Fig. 4. Diagrammatic representation of a paper chromatogram of indole amines prepared from urine of a schizophrenic patient.

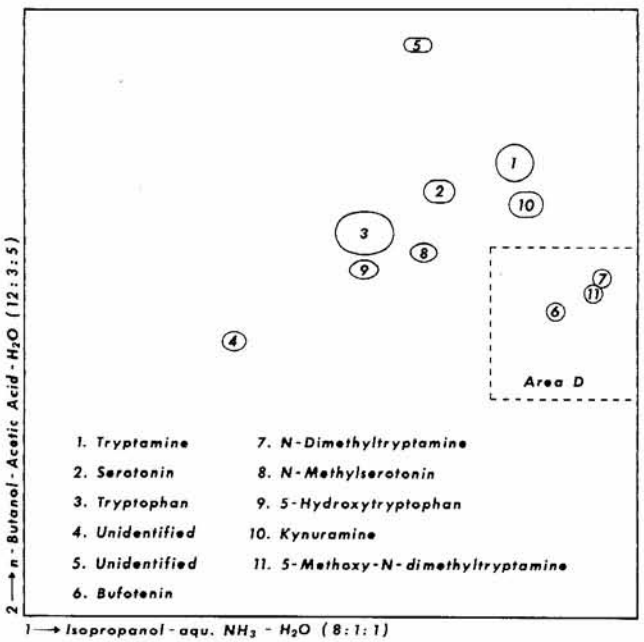

Fig. 5. Diagrammatic representation of a thin-layer chromatogram of indole amines prepared from urine of a schizophrenic patient.

mine and serotonin, primary amines, did not give peaks at the position of their Schiff bases until after the acetone reaction. The bufotenin peak, however, showed no change in location before or after the acetone reaction (Fig. 6); this implies that this compound did not contain primary amino groups. Some samples were further purified by preparative thin-layer chromatography, being extracted from Silica Gel G at area D (Fig. 5). This purified sample also gave the bufotenin peak on gas–liquid chromatograms.

Thin-layer chromatography revealed spots suspected of being N-methylserotonin, N,N-dimethyltryptamine, and/or 5-methoxy-N,N-dimethyltryptamine in some patients on the MAO inhibitor. The presence of these amines, however, was not confirmed by the gas–liquid method due to the appearance of large neighboring peaks, probably as a result of impurities in the sample.

The amount of bufotenin excreted in the urine was calculated from the size of the peak appearing on gas–liquid chromatograms. In the absence of MAO blockade, the total excretion of bufotenin was estimated as being less than 1 μg per 24 hr; in the presence of the MAO inhibitor, the value rose to 2–5 μg a day for both the free and conjugated forms.

It is interesting to correlate these biochemical findings with the clinical observations of the mental symptoms. In all of our patients, the psychotic symptoms became uniformly worse about two weeks after an

increase of urinary bufotenin started, and the aggravation of psychotic symptoms continued as long as the higher rate of bufotenin excretion continued.

DISCUSSION

The wide distribution of bufotenin and N,N-dimethyltryptamine in higher plants has been reported by some investigators [32]. We carefully excluded, however, all known sources of preformed catechol and

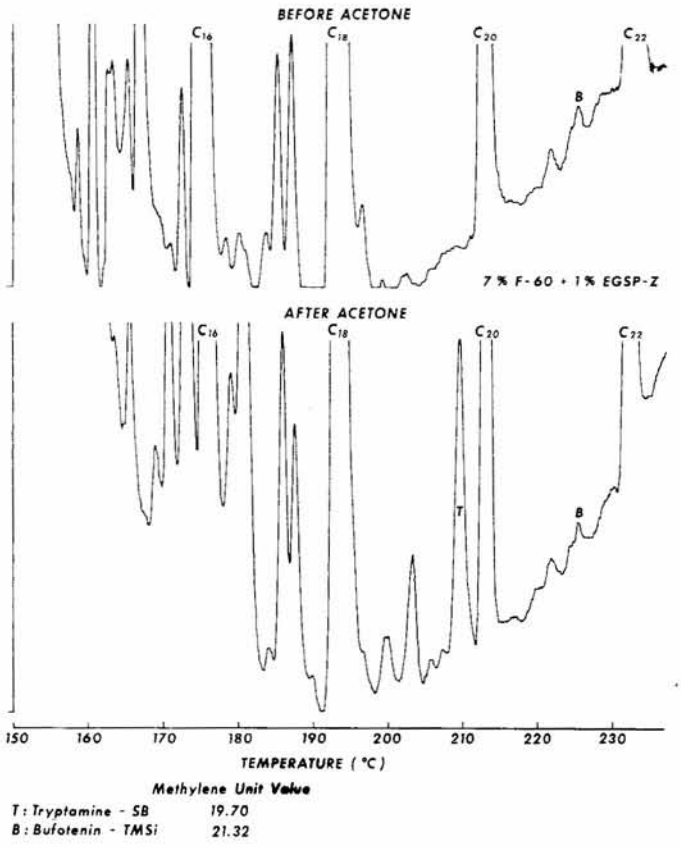

Fig. 6. Gas–liquid chromatograms of urinary amines from a schizophrenic patient (SP). Both chromatograms were obtained from the same sample injected with standard even-numbered straight-chain saturated hydrocarbons (C_{16} to C_{22}). All hydroxyl groups were converted to trimethylsilyl ether groups. The sample in the lower portion of the figure was injected after being reacted with acetone where primary amines were converted to eneamines. (T) Acetone condensation product of tryptamine; (B) trimethylsilyl ether of bufotenin. The peak due to the acetone condensation product of the trimethylsilyl ether of "serotonin" was deleted from the bottom chromatogram because it appeared late, after the temperature had reached the upper limit of acceptability (230°C).

indole amines from the diet of our patients. Therefore, it seems that the N,N-dimethylated indole amines found in the urine in the present study formed in the bodies of our patients rather than came from exogenous sources, inasmuch as the presence of the enzyme catalyzing N-methylation of indole amines in mammalian tissues has been reported by Axelrod [6, 7].

On the basis of the above-mentioned correlation between biochemical and clinical observations and the fact that N,N-dimethylated indole amines evoke psychotomimetic effects in human beings [1–4], it would seem that the worsening of psychotic symptoms in our schizophrenic patients could be partially mediated by elevated levels of such tertiary indole amines, as suggested by Brune and Himwich [9], at least under the present experimental conditions.

Our tentative explanation of possible biochemical mechanisms involved in the psychotogenic effects of certain amino acids on schizophrenic patients in the presence of MAO blockade is shown in Fig. 7. In our opinion, the level of free N,N-dimethylated indole amines plays a role in this phenomenon. The maintenance of elevated levels of free tertiary amines in the body fluids may exacerbate mental and behavioral symptoms. The levels of these toxic amines are determined by the dynamic balance between the process of their synthesis and detoxication. Any factor which can cause an increased concentration of these amines may act psychotomimetically on schizophrenic patients. The amino acids listed in Fig. 7 have been found to exacerbate schizophrenic symptoms when given excessively in the presence of a MAO inhibitor [8–14,

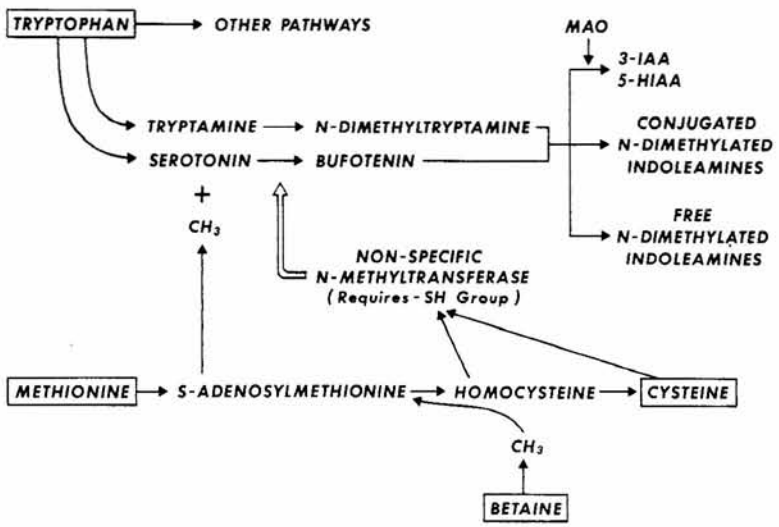

Fig. 7. Possible biochemical pathways for formation and detoxication of N,N-dimethylated indole amines in schizophrenic patients. (MAO) monoamine oxidase; (3-IAA) indole-3-acetic acid; (5-HIAA) 5-hydroxy-indoleacetic acid.

29]. Each of these amino acids can, in its own way, facilitate the formation of psychotogenic N,N-dimethylated indole amines. Tryptophan provides the tryptamine structure which can serve as a precursor of tertiary amines, while methionine or betaine, methyl donors, can supply methyl groups. Cysteine and methionine may make thiol groups available for the activation of the enzyme catalyzing the formation of N,N-dimethylated indole amines [7]. The amount of free tertiary indole amines may also increase when the metabolic breakdown process is blocked by a MAO inhibitor. Thus, the experimental results reported can be explained along these lines.

The amounts of psychotogenic N,N-dimethylated indole amines found in the urine are much smaller than those used in the pharmacological experiments in order to evoke psychotomimetic effects in human beings [1–4]. It should be remembered, however, that these pharmacological experiments used a single, acute dosage and that toxic effects were observed in addition to psychopharmacologic ones. In our patients, exacerbation of the symptoms occurred about two weeks after the first elevation of free tertiary indole amines. We, therefore, feel that long-term action of small amounts of free tertiary indole amines may be necessary to evoke psychotic symptoms in schizophrenic patients. We cannot answer at present, however, the question as to whether or not a similar biochemical mechanism is operative in the pathogenesis of schizophrenia because of lack of sufficient evidence, although we hope to attain some such evidence from the schizophrenic patients we now have under investigation. In addition, preliminary studies on the urine samples of our four mental defectives showed that one of these four, who exhibited psychotic symptoms, excreted free bufotenin.

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