Urinary Acid Metabolites of Biogenic Amines in Schizophrenic Patients

by Edward F. Domino

Abstract

A hypothesis is presented that only pure type B monoamine oxidase (MAO) amine substrates and their oxidized metabolites should be altered in mental patients, especially paranoid chronic schizophrenics. If urinary pH is rigorously controlled, it is predicted that 24-hour urinary amine levels (free and total) of pure type B MAO substrates will be elevated and their corresponding acid metabolites reduced, especially under excess substrate conditions in vivo. The usual 50 percent decrease in platelet MAO, which is reported to be low in some schizophrenic patients in vitro, should especially show biochemical functional deficits in vivo when the organism is stressed with a suitable amine or amino acid substrate given as a diagnostic load. Such an approach may be useful in demonstrating a functional biochemical deficit and the biological significance of low platelet MAO activity (a type B MAO) seen in some psychiatric patients.

Many investigators would probably agree with Wyatt and Murphy (1976) that the evidence that some schizophrenic patients have reduced platelet MAO activity is impressive. Yet, in disagreement, Youdim (1979) has suggested that “the situation at present is rather confusing and controversial because there is no agreement” (p. 89). The fact that some studies are negative does not negate the positive findings. Obviously, further studies, using new approaches, are needed. Belmaker and Ebstein (1979) have suggested a “sophisticated biochemical examination of human platelet MAO, as to Michaelis constant, electrophoretic mobility at different pH levels, sensitivity to many inhibitors... both in normal and behaviorally disturbed subjects” (p. 100). The purpose of this report is to suggest still another direction for future research to test the biochemical functional significance of a reduced type B MAO in platelets and possibly other peripheral tissues of the mentally ill.

After the initial reports of Murphy and Wyatt (1972) and Wyatt et al. (1973), we were able to confirm their findings using platelets from unmedicated chronic schizophrenic patients in which 14C-tryptamine (14C-T) was used as substrate (Domino and Khanna 1976). Subsequently, we also showed that the in vitro half-life of 14C-T in the whole blood of similar chronic schizophrenic patients was prolonged (Domino and Gahagen 1977). Youdim (1979) pointed out that humans with iron deficiency have significantly reduced platelet MAO activity. Rats made iron deficient show a similar decrease of MAO activity in platelets but not in brain. Although iron deficiency may explain some forms of reduced platelet MAO, it does not appear to apply to our small series of schizophrenic patients because they were on prophylactic vitamin and mineral (including iron) therapy at the time their platelet MAO activity was measured. These patients had normal hemoglobin and hematocrit levels.

A key point is that (in our own studies as well as those in the literature) platelet MAO activity is seldom reduced more than 50 percent below normal. Yet it is generally agreed by most biochemists that for a functional enzyme deficit one needs at
least an 80 to 90 percent reduction of enzyme activity. This apparently is true for the MAO enzymes as well. The use of tranylcypromine (an irreversible nonspecific MAO inhibitor) and 1-tryptophan or L-dopa treatment in rats produces two different types of motor hyperactivity syndromes. Youdim (1979) reported that 85 percent of rat brain MAO activity must be inhibited before a hyperactivity syndrome occurs to either amino acid treatment. Youdim has also shown that the platelet MAO activity of patients who were nonresponders to 5-hydroxytryptophan plus deprenyl was more than 20 percent of normal. If a figure of 80 percent inhibition of MAO activity is necessary for a functional biochemical deficit, then one would be forced to conclude that all studies (in which platelet MAO activity is only at best 50 percent reduced) indicate that there is no functional disturbance of MAO in mental patients. It is generally agreed that human platelet MAO is of the B type. In addition, human skeletal muscle MAO is of the B type. Schizophrenic patients also have reduced skeletal muscle MAO activity, as described by Meltzer, Jackman, and Arora (1980; this issue). I believe that it is essential to show that these reported reductions in type B MAO activity in some mental patients, especially schizophrenics, are functionally relevant.

As pointed out by Youdim (1979), rat brain MAO activity is quite resistant to change. Brain MAO in autopsy material from chronic schizophrenics normally oxidizes 14C-T (Domino, Krause, and Bowers 1973). It should be noted that brain has both type A and type B MAO, and both are apparently normal in schizophrenic patients. Youdim's finding that rat brain MAO is more "resistant" to change implies that human brain MAO may be different from that in other tissues as well.

It is extremely important to realize that some type B substrates in vitro act as type A substrates in vivo. For example, tryptamine (T) is a mixed type A and B substrate but a type A substrate when given in vivo intraventricularly to rats (Neff et al. 1974). This is probably true of many other amines with mixed type A and B MAO affinities. Furthermore, there are important species differences. For example, dopamine (DA) may act as a type A substrate in the rat and a type B substrate in man.

In view of these facts, it seems logical to hypothesize that biogenic amines that are oxidized predominantly by type A MAO will have normal acid metabolite levels, while those that are oxidized predominantly by type B MAO will have reduced acid metabolites in patients with low platelet MAO. Depending on what specific tissues and substrates are examined, one would expect widely varying results. Obviously, in living humans the tissues and fluids which can be examined are quite limited. One really can easily examine only blood, urine, and cerebrospinal fluid. For reasons of convenience in our laboratories, both at the Lafayette Clinic and at the University of Michigan, we have been examining urinary metabolites of neurotransmitters. Such an approach has obvious limitations, but also some advantages in that the patient is treated as a whole.

The urinary consequences of the relative type B MAO deficit are summarized in figure 1. In this scheme the entire body is viewed as a funnel. The various amine substrates are then exposed to a variety of tissue MAOs, including blood, brain, skeletal muscle, and many other tissues. All compounds must pass through the kidneys in order to appear in the urine as either free or conjugated amines or their oxidized metabolites. The pure type A substrates are represented by noradrenaline (NE) and 5-hydroxytryptamine (5HT). The mixed type A and B substrates are represented by phenylethylamine (PEA), phenylethanolamine (PEOA), and tele-methylhistamine (tMH).

As new amines and their substrate MAO specificity are determined, they could be added conveniently to each category. The specific predictions that one can make from the hypothesis that a type B MAO, in contrast to a type A MAO, functional deficit exists are many. One should collect 24-hour urine samples and assay the total levels of free and conjugated amines and their metabolites. Urinary pH is very important in the amount in which weakly acidic and basic drugs are excreted.

Price (1975, 1976) showed the dependence of T excretion on urinary pH. Both Reynolds et al. (1978) and Aslan et al. (1979) found a relatively weak negative correlation between urinary pH and epinephrine (EPI) excretion. However, Aslan et al. showed that neither mean NE nor EPI excretion differed with a urinary pH change from 5.78 to 7.44. Hence, it is important to know the pH of each neurotransmitter and metabolite of interest to determine the precise role of urinary pH on mean 24-hour excretion. Assuming that urinary pH is controlled, one can make a number of interesting predictions regarding the 24-hour urinary amine and major metabolite levels in schizophrenic patients with low type B MAO. One would expect that with normal type A MAO activity there
Figure 1. Hypothesized urinary chemical consequences of normal type A and reduced type B MAO activity in mental patients

The body is viewed as a funnel through which the various amine substrates percolate. Symbols are as follows:

- NE - norepinephrine
- 5HT - 5-hydroxytryptamine, serotonin
- DA - dopamine
- T - Tryptamine
- PEA - phenylethylamine
- PEOA - phenylethanolamine
- tMH - tele-methylhistamine
- HMPG - hydroxymethoxyphenylglycol also called methoxyhydroxyphenylglycol

**Predictions:**

- No change; ↑ Increase; ↓ Decrease

would be normal urinary levels of NE, its major metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG), 5HT, and its major metabolite 5-hydroxyindoleacetic acid (5HIAA). One would have to know how mixed type A and B substrates in vitro act in vivo in man. In view of the evidence that there is predominant in vivo oxidation by type A MAO, then it also follows that urinary DA and T levels should be normal in
schizophrenic patients, as would their respective acid metabolites homovanillic acid (HVA) and indoleacetic acid (IAA).

In sharp contrast to the above amines and their oxidized metabolites, the type B MAO amine substrates should be elevated in schizophrenic patients and their acid metabolites reduced if most type B MAO in the body is functionally reduced. Hence, urinary PEA, PEOA, and tMH should be elevated and their respective oxidized products phenylacetic acid (PAA), phenylethanolacetic acid (POAA), and tele-methylimidazoleacetic acid (tMIAA) reduced. This would especially be true if excessive substrates were available, as would be the case in loading experiments. Precursor loading experiments have been widely used and have obvious uses and limitations (Curzon 1979). Attempts to "stress" the biochemical system with a precursor load have proven to be a valuable approach. This technique has been used, for example, in the patient with diabetes given a glucose load.

Many investigators have used tryptophan loads in psychiatric patients. Much of the early research has been reviewed by Eiduson et al. (1964). As might be expected, it is important to know whether pure L- or racemic DL-tryptophan was used, for the results vary markedly. Zeller et al. (1957) and Lauer et al. (1958) reported that .1 mmole/kg of L-tryptophan given orally caused a 100 percent increase in 5HIAA excretion in normal subjects but not in schizophrenic patients. However, when Banerjee and Agarwal (1958) used 5 g loads of DL-tryptophan, they observed that schizophrenic patients had a two-fold increase in urinary 5HIAA, whereas normal controls did not. Both groups excreted more urinary IAA after the racemic tryptophan loads. Urinary kynurenine and 3-hydroxyanthranilic acid were absent in the schizophrenic patients but present in the normal subjects. Wachstein and Lobel (1956) earlier reported that after vitamin B6 administration schizophrenic patients given 4-methyltryptophan showed the normally expected increase in xanthurenic acid but no increase before B6. It would appear that the schizophrenic patients they studied had a B6 deficiency. That might be anticipated because of the diets many psychiatric patients were fed at that time before recognition that many psychiatric patients were malnourished. Inasmuch as kynurenine is a mixed type A and B substrate, it would be of interest to know whether schizophrenic patients with adequate vitamin intake have altered urinary kynurenine and xanthurenic levels. Brown, White, and Kennedy (1960) found no differences in the urinary excretion of many tryptophan metabolites in schizophrenic patients. However, N-methyl-2-pyridone-5-carboxamide was reported to be reduced.

Kopin (1959) reported that urinary 5HIAA levels are similar in schizophrenic patients and normal controls before and during a 5 g L-tryptophan load. Sprince et al. (1961) reported excessive excretion of urinary indoleacetamide in schizophrenic patients. This compound may have arisen from indoleacetylglucuronic acid with their isolation procedures. Even if this is the case, then indoleacetylglucuronic acid excretion may be in excess in such patients.

Weissbach et al. (1959) found normal urinary values of free and total IAA in schizophrenic patients. Century, Ahlberg, and Vogel (1968) studied the urinary excretion of tryptophan, T, and IAA before, during,

and after oral tryptophan in male normal subjects and schizophrenic patients. They showed that a 15 mg/kg oral tryptophan load increased IAA acid excretion significantly more in the schizophrenic subjects, whereas the increases in urinary tryptophan and T were not significantly different. Hence, they could find no evidence of a functional deficiency in the oxidation of T. In fact, just the opposite was observed in that urinary IAA levels were enhanced in the schizophrenic patients.

One criticism of many of the previous studies is that relatively nonspecific chemical assay procedures were used to study the neurotransmitters and their metabolites. We decided to use relatively specific gas chromatographic-mass spectrometric analyses with selected positive ion monitoring. HVA, 5HIAA, and IAA were assayed as described by Domino, Mathews, and Tait (in press). Urinary creatinine (C) was assayed using the HPLC assay of Buchanan, Tait, and Domino (1979). Urinary free T was isolated as per the method of Christian et al. (1975). Table 1 summarizes the 24-hour urinary levels of HVA, IAA, 5HIAA, and T, expressed per mg C in seven control subjects and seven chronic, drug-free schizophrenic patients. In addition, the weight, body surface, 24-hour urinary volume, urinary pH, and T/IAA ratio were measured. These data will be reported elsewhere in detail (Mathews, Tait, and Domino, in preparation). LaBrosse et al. (1964) have pointed out the value of a urinary T/IAA ratio as an index of monoamine oxidase inhibition. Only if urinary pH is controlled is their suggestion valid.

It can be noted in our small sample of normal controls and chronic, drug-free schizophrenic patients that the patients weighed less, had a
Table 1. Comparison of 24-hour urinary levels of homovanilllic, indoleacetic, 5-hydroxyindoleacetic acid, and tryptamine levels in drug-free chronic schizophrenic patients and mentally normal control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
<th>Probability</th>
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<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>71.4</td>
<td>65.5</td>
<td></td>
</tr>
<tr>
<td><strong>Body surface area (m)</strong></td>
<td>1.92</td>
<td>2.01</td>
<td></td>
</tr>
<tr>
<td><strong>24-hour volume (ml)</strong></td>
<td>1381</td>
<td>1320</td>
<td></td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>5.7</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td><strong>C (mg/kg·1)</strong></td>
<td>20.3</td>
<td>21.3</td>
<td></td>
</tr>
<tr>
<td><strong>HVA (µg/mg·C)</strong></td>
<td>8.23</td>
<td>4.40</td>
<td></td>
</tr>
<tr>
<td><strong>SHIAA (µg/mg·C)</strong></td>
<td>21.3</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td><strong>T (ng·mg⁻¹·C)</strong></td>
<td>16.3</td>
<td>16.3</td>
<td></td>
</tr>
<tr>
<td><strong>T/IAA (x 10⁻³)</strong></td>
<td>6.52</td>
<td>3.02</td>
<td></td>
</tr>
<tr>
<td><strong>Probability</strong></td>
<td>0.021</td>
<td>0.029</td>
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HVA = homovanilllic acid.
SHIAA = 5-hydroxyindoleacetic acid.
IAA = indoleacetic acid.
C = creatinine.
T = tryptamine.

Student's t-test group comparison, two-tailed estimates of probability.
smaller body surface area, and excreted less urine. In each case, group comparison Student's t tests were highly significant (p < .02, < .03, < .003, respectively). It was not surprising that 24-hour urinary volume was reduced in the patients, for the drug-free chronic schizophrenics were very uncooperative. There was no significant difference in urinary pH, C, HVA, 5HIAA, and T levels or in the urinary T/1AA ratio. The levels of 1AA were significantly higher in the schizophrenic patients (p < .01) but this is certainly of no practical diagnostic significance. Hence, one would conclude that baseline 24-hour urinary excretion of these neurotransmitter metabolites does not vary significantly from that in controls. This, of course, would be expected if only type B MAO activity were reduced functionally in these schizophrenic patients.

Instead of examining type A or mixed type A and B MAO substrates and metabolites in the urine, one should examine only pure type B MAO amine substrates and products in schizophrenic patients and others who show low platelet MAO activity. Such research is already in progress. Potkin et al. (1979) have reported that urinary PEA, a pure type B MAO substrate, is increased in paranoid chronic schizophrenic patients as compared to nonparanoid schizophrenic and normal control subjects. Inasmuch as platelet and lymphocyte MAO is reduced in chronic schizophrenic patients with paranoid symptoms (Demisch et al. 1977; Wyatt, Potkin, and Murphy 1979), it would appear that the logic of the selective type B substrate and metabolite hypothesis proposed is fruitful. It follows that PAA urinary levels should be reduced in the same patients. Our own approach is to examine urinary tMH and tMIAA levels in similar patients, for tMH is a selective type B MAO substrate (Domino 1980; Hough and Domino 1979).

If excessive type B MAO substrates are administered, one should be able to show even greater functional biochemical deficits, as predicted in figure 1. It is to be hoped that this hypothesis will be tested for all pure type B MAO substrates and their oxidation products. One complication that must be considered in this type of research is the role of bacterial flora in the gastrointestinal tract. It is well known that bacterial metabolism contributes to the levels of various amine metabolites, especially IAAs (Weissbach et al. 1979). Hence, when highly significant changes are found, it is necessary to use oral chemotherapy to sterilize the gastrointestinal tract in order to show that the differences observed are related to the human organism and not gastrointestinal bacteria.

References


Domino, E.F.; Mathews, B.; and Tait, S.K. Urinary transmitter metabolites in drug-free chronic schizophrenic patients measured by gas
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