Reduced Catechol-O-Methyltransferase Activity in Red Blood Cells of Women with Primary Affective Disorder

Abstract. *Red blood cell catechol-O-methyltransferase, histamine-N-methyltransferase, and a methanol-forming enzyme were examined in a number of subjects with mental diseases. Catechol-O-methyltransferase activity was significantly reduced in female subjects with primary affective disorder (depression) as compared to normal women and men, men with primary affective disorder, and schizophrenic men and women. In depressed women, histamine-N-methyltransferase activity was elevated and the methanol-forming enzyme was unchanged.*

In recent years there have been indications of biological differences in subjects with affective illness (depression). It has been proposed that there is a defect in biogenic amine metabolism, particularly with regard to catecholamines in these patients (*1*). Support for such a hypothesis is the demonstration that drugs which affect uptake and metabolism of catecholamines are also therapeutically effective in these disorders (*2*). Recent work in our laboratory has shown that catechol-*O*-methyltransferase (COMT), a major enzyme in the metabolism of catecholamines (*3*), as well as other methyltransferase enzymes, histamine-*N*-methyltransferase (*4*) and a methanol-forming enzyme (*5*), are present in human red blood cells (*6*). The availability of red blood cells provided an opportunity to examine the activity of these enzymes in a variety of physical and mental diseases in man. In women with primary affective illness a significant decrease in the activity of COMT in red blood cells and a significant increase in histamine-*N*-methyltransferase were observed.

Diagnosis of primary affective disorder was made according to the criteria of Winokur and Clayton (*7*). This included patients with a history of mania, depression, or both, provided that there was no preexisting medical or other psychiatric illness which may be associated with depression. Blood samples were obtained from subjects with primary affective disorder who were hospitalized at the Clinical Center, National Institutes of Health, Bethesda, Renard Hospital, St. Louis, and St. Elizabeths Hospital, Washington, D.C., or were outpatients at these centers. Schizophrenic patients had been chronically ill for at least 3 years with evidence of hallucinations or delusions, or both, insidious onset, and a progressive course without return to premorbid function ("nuclear schizophrenia"). No patients had a primary diagnosis of alcoholism. Control samples were obtained from randomly selected, medically ill patients from the above hospitals and from normal volunteers. Care was taken to exclude as controls subjects with a history or family history of psychiatric illness, although some of the medically ill patients had depression associated with their illness ("secondary affective disorder"). Blood samples were drawn at various times during the day and assayed immediately or stored at −80°C for up to 5 days.

For enzyme assays, heparinized blood was centrifuged at 2000*g* at 4°C for 10 minutes and the plasma was removed by aspiration. The red blood cells were lysed by diluting a 1.0-ml portion to 10 ml with ice-cold water. After the lysed cells were left on ice for 10 minutes, they were centrifuged at 2000*g* for 10 minutes at 4°C to remove the ghost fraction. The supernatant was used to assay all three enzymes from a single specimen of blood. A 0.5-ml portion was taken for enzyme assay (*6*). Histamine-N-methyltransferase, COMT, and the methanol-forming enzyme were measured by a modification of methods previously described (*3–6*). The ^{14}C-methylated metabolities were extracted into 3 ml of isoamyl alcohol. To assay the methanol-forming enzyme, 1.0-ml portions of the isoamyl extract were transferred to two separate counting vials. The radioactivity was measured directly in one vial after the addition of ethanol (1 ml) and phosphor (10 ml). The isoamyl extract in the other vial was evaporated to dryness in a chromatography oven at 80°C and the radioactivity was measured after the addition of isoamyl alcohol (1 ml), ethanol (1 ml), and phosphor (10 ml). For the COMT and histamine-*N*-methyltransferase assay, a 2-ml portion of the isoamyl alcohol extract was transferred to a vial and evaporated as above. The remaining [^{14}C]normetanephrine or [^{14}C]methylhistamine were measured after the addition of ethanol (1 ml) and phosphor (10 ml). A blank was obtained by incubation of the reaction mixture without substrate and treating as above.

There was a significant reduction in COMT activity in red blood cells of women with primary affective illness as compared to controls of either sex or men with primary affective disorder (Fig. 1 and Table 1). Although there was a highly significant difference, some of the values from patients with affective illness were in the normal range. Histamine-*N*-methyltransferase activity in red blood cells showed a significant elevation in women with primary affective illness as compared to normal women (see Table 1). The methanol-forming enzyme in the red blood cells was

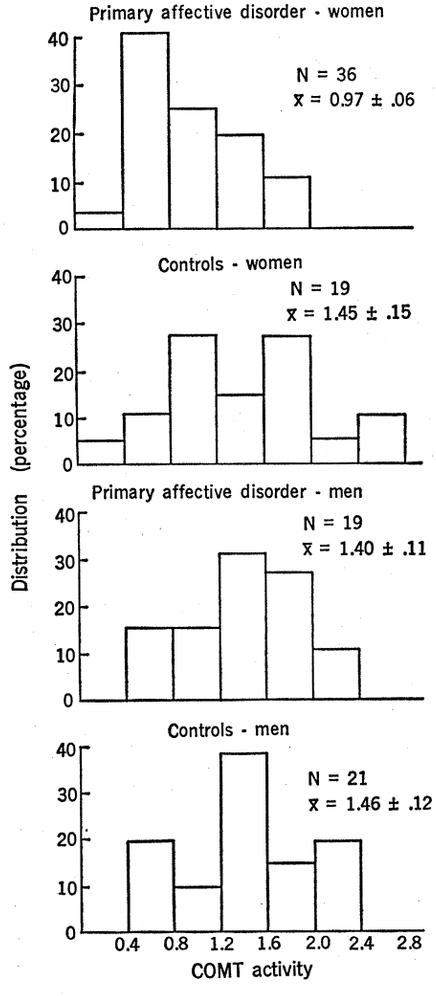

Fig. 1. Distribution of COMT activity in red blood cells in male and female patients with primary affective disorder and in controls. Results are expressed as the number of nanomoles of [^{14}C]-normetanephrine formed per milliliter of red blood cells per hour after incubation with [^{14}C]*S*-adenosylmethionine and norepinephrine with red blood cell hemolysates.

Table 1. Activities of methyltransferase enzymes in red blood cells of women and men with primary affective disorder (depression) and in control men and women. Activities are expressed as the number of nanomoles of the ^{14}C product formed per milliliter of red blood cells per hour. Values are the mean ± S.E.M. Numbers in parentheses are the number of subjects.

Subject	Catechol-*O*-methyltransferase	Histamine-*N*-methyltransferase	Methanol-forming enzyme
	Women		
Depression	0.97 ± .06* (36)	0.29 ± .03† (11)	0.46 ± .08 (11)
Control	1.45 ± .15 (19)	0.18 ± .01 (7)	0.41 ± .06 (7)
	Men		
Depression	1.40 ± .11 (19)	0.27 ± .04 (10)	0.41 ± .08 (9)
Control	1.46 ± .12 (21)	0.21 ± .01 (14)	0.33 ± .02 (14)

* $P < .01$ as compared to control women; $P < .001$ compared to men with primary affective disorder; and $P < .001$ compared to control men. † $P < .02$ compared to control women (Student's *t*-test).

not different among the various subjects.

To determine whether patients with other mental diseases showed similar changes, COMT was examined in 5 schizophrenic women and 13 schizophrenic men. Mean COMT activity in red blood cells of schizophrenic women was 1.52 ± 0.12. This was not statistically different from values of control subjects but was different when compared to women with affective illness ($P < .01$). Similarly, schizophrenic men had a mean activity of 1.52 ± .16, statistically different from depressed women ($P < .001$). The other transferase enzymes were not examined in these subjects.

To examine the possibility that these differences might be due to environmental factors, blood samples were obtained from depressed subjects in an outpatient clinic at NIH as well as from the two other psychiatric centers. The values from these depressed subjects showed the same decrease in COMT activity as compared to normal subjects from the same centers.

Certain physiochemical properties of red blood cell COMT were examined in depressed women and in normal women. These included *p*H optimum, initial activity, Michaelis constant (K_m), and linearity of reaction with respect to dilution and time. The initial velocity was proportionally lower in women subjects with primary affective illness. Red blood cell COMT from both groups showed linearity with dilution and time up to 60 minutes incubation. The K_m and *p*H optimum were similar. To examine for the presence of an enzyme inhibitor of COMT, lysed red blood cells were dialyzed overnight in Ringer's solution. They showed no increase in activity. Furthermore, the mixing of enzyme preparations from patients with low values and subjects with normal values showed additive enzyme activity.

Repeated analyses of several patients and normal subjects over 6 months showed consistent values (within 10 percent). Values for COMT activity were also obtained from depressed patients before and after various treatments, including tricyclic antidepressants, lithium carbonate, L-dopa (dihydroxyphenylalanine), α-methyl-*p*-tyrosine, *p*-chlorophenylalanine, and electroconvulsive therapy, without any change in values. Clinical improvement in depressed patients did not change the activity of COMT, and clinically well outpatients or patients obtained during a follow-up study had the same results as severely depressed hospitalized patients. Values in patients during a manic episode were not different from those from the same patients during depression.

Red blood cell indices from depressed patients were normal. No relationship occurred between subjects with abnormal menses and altered COMT values. The activity of COMT did not vary with age of patients or control subjects.

Although the significance of altered COMT and histamine-*N*-methyltransferase activity in women with affective illness but not in men with the same illness is unclear, the results suggest a possible sex hormonal association. Consistent with this is the observation that women but not men become more responsive to antidepressant drugs after thyroid administration (*8*). Other factors which are directly unrelated to depression, but may be secondary to an altered environmental factor, such as diet, abnormal menses, or medication, do not appear as likely reasons for these differences.

It has been proposed that the biological defect in depression is a deficiency in catecholamines (*1*). This defect can be due either to reduced release of catecholamines from nerve endings and adrenal gland or a reduced responsiveness of the adrenergic receptor. Drugs which are therapeutically useful in depression make more noradrenaline available to the receptor by preventing inactivation of the catecholamine through reuptake into the nerve or by inhibiting its metabolism by monoamine oxidase.

The enzyme COMT is closely associated with adrenergic receptors with respect to substrate specificity and association with the effector cell (*9*). It is possible that red blood cell COMT activity might reflect activity of the adrenergic receptor and that the lower activity in depressed women suggests reduced activity of adrenergic receptors. Another possibility is that this enzyme might serve to inactivate noradrenaline discharged into the circulation and this decreased activity might represent a compensatory mechanism for making more physiologically active amine available to receptors. An elevated amount of plasma noradrenaline (*10*), an increased excretion of amine products (*11*), and a decreased responsiveness to injected noradrenaline in depressed subjects (*12*) support this hypothesis.

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