# Catechol-*O*-Methyltransferase: Thermolabile Enzyme in Erythrocytes of Subjects Homozygous for Allele for Low Activity

Abstract. *Low catechol-O-methyltransferase (COMT) activity (< 8 units per milliliter) in the human erythrocyte is inherited as an autosomal recessive trait* ($COMT^L$). *The average half-life of COMT in erythrocyte lysates incubated at 48°C was significantly shorter in lysates from three subjects with low enzyme activity than in lysates from three subjects with high enzyme activity (12.5 ± 0.9 minutes compared with 21.2 ± 1.4 minutes,* $P < .01$). *When the ratios of COMT activities in lysates heated at 48°C for 15 minutes to enzyme activities in unheated samples were used as a measure of enzyme thermostability in blood samples from 316 randomly selected subjects, the ratios were significantly less for subjects with low enzyme activity than for subjects with higher enzyme activity. The presence of thermolabile COMT in blood of individuals homozygous for* $COMT^L$ *raises the possibility that the locus* COMT *may represent the structural gene for the human enzyme.*

Catechol-*O*-methyltransferase (E.C. 2.1.1.6; COMT) plays an important role in the metabolism of the endogenous catecholamines epinephrine, norepinephrine, and dopamine (*1*) and in the metabolism of catechol drugs used in the treatment of patients with hypertension, asthma, and Parkinson's disease (*2*). The enzyme is present in many tissues including the erythrocyte (*1, 3*). The frequency distribution of erythrocyte COMT activity values in a randomly selected population is bimodal and includes a subgroup of approximately 25 percent of subjects with low enzyme activity (< 8 units per milliliter of erythrocytes) (*4*). The trait of low erythrocyte COMT activity (< 8 unit/ml) is inherited in an autosomal recessive fashion, and the allele for low erythrocyte enzyme activity has a gene frequency of approximately .5 (*5*). The relative COMT activity in human blood is directly and positively correlated with the relative enzyme activity in the human lung and kidney (*6*), an observation that raises the possibility that the measurement of COMT activity in blood may give information with regard to individual variations in the *O*-methylation of endogenous catecholamines and of catechol drugs.

The difference in enzyme activity between subjects with genetically low and with genetically high erythrocyte COMT activity is not a result of different concentrations of endogenous enzyme inhibitors or activators (*4*). However, genetically determined differences in the biochemical properties of the enzyme might account for the differences in COMT activity. In the experiments described here we compared the biochemical properties of erythrocyte COMT in the blood of subjects with high and with low enzyme activity.

Blood samples for an initial series of biochemical studies were obtained from three subjects with erythrocyte COMT activity of less than 8 units per milliliter of red blood cells and three subjects with enzyme activity much greater than 8 unit/ml. All of these subjects were selected from a previously studied population (*5*). Blood samples were also obtained from 316 randomly selected unrelated blood donors at the Mayo Clinic Blood Bank. All blood donors were white and none of them were taking any medication at the time the blood samples were obtained. Erythrocyte COMT activity was measured by the method of Raymond and Weinshilboum (*7*), a procedure that includes a step in which calcium, an inhibitor of COMT (*8*), is removed from the lysate. Rat liver COMT was partially purified as described (*7*). Michaelis-Menten ($K_m$) values were calculated by the method of Wilkinson with a Fortran program written by Cleland (*9*).

The average erythrocyte COMT activity in lysates from the three subjects with low activity was 6.0 ± 0.3 unit/ml (range 5.4 to 6.3) and from the three subjects with high activity was 19.2 ± 2.4 unit/ml (range 15.1 to 23.4). Maximum enzyme activity for both groups of samples occurred at a *p*H between 7.7 and 8.1. There were no significant differences between the two groups in apparent $K_m$ values for either of the cosubstrates of the reaction [dihydroxybenzoic acid (DBA) and *S*-adenosyl-l-methionine (SAM)] or for the enzyme activator, magnesium. The apparent $K_m$ values for the low- and high-activity groups, respectively, were $3.49 \pm 0.47 \times 10^{-6} M$ [mean ± standard error (S.E.)] and $3.85 \pm 0.35 \times 10^{-6} M$ for SAM; $1.40 \pm 0.10 \times 10^{-5} M$ and $1.56 \pm 0.14 \times 10^{-5} M$ for DBA; and $1.08 \pm 0.06 \times 10^{-4} M$ and $1.20 \pm 0.13 \times 10^{-4} M$ for magnesium. In addition, there were no differences between the two groups in the concentrations of three different COMT inhibitors, tropolone, *S*-adenosyl-l-homocysteine, and calcium (*8, 10*), at which a 50 percent inhibition of the enzyme occurred.

However, when the thermostability of a lysate from one subject with very low enzyme activity and one with very high enzyme activity were compared by incubation of lysates in a water bath for 15 minutes at various temperatures be-

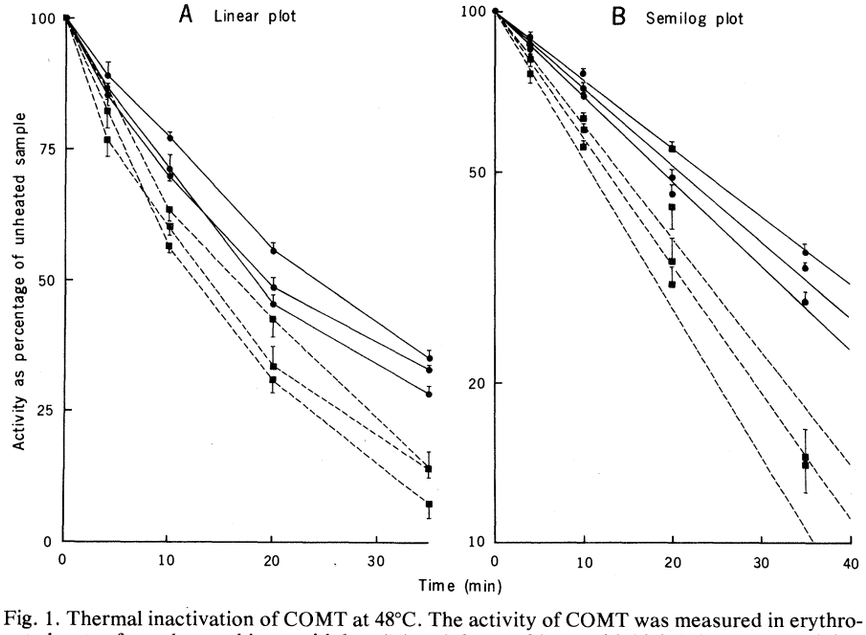


Fig. 1. Thermal inactivation of COMT at 48°C. The activity of COMT was measured in erythrocyte lysates from three subjects with low (■) and three subjects with high (●) enzyme activity, after the lysates were incubated at 48°C for various time periods. (A) The data are plotted in a linear fashion. Each point represents the mean (± standard error) of three determinations. (B) The data are plotted in a semilogarithmic fashion. The results for each subject have been joined by a linear least-square line.


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tween 43° and 53°C, the low-activity sample was 50 percent inactivated at 47.8°C, whereas the high-activity sample was 50 percent inactivated at 48.9°C. Because of this observation, and because relative thermal stability is a sensitive indicator of differences in protein structure (*11*), lysates from each of the six subjects were incubated at 48°C for variable periods of time and COMT activity was measured (Fig. 1). The average half-life of COMT in the lysates from the subjects with low COMT erythrocyte activity was 12.5 ± 0.9 minutes (mean ± S.E.), whereas that for lysates from high-activity subjects was 21.2 ± 1.4 minutes ($P < .01$). When the data shown in Fig. 1 were examined in a slightly different fashion, the average slope of the thermal inactivation curves for the low enzyme activity group, −.0268 ± .0029 log percentage per minute (mean ± S.E.), was significantly greater than that for the subjects with higher basal enzyme activity, −.0142 ± .0009 ($P < .02$).

Because these differences in COMT thermostability might reflect differences in the thermostability of endogenous enzyme inhibitors or activators, partially purified rat liver COMT was added to lysates both before and after they were incubated at 48°C. The recovery of rat liver COMT added to lysates prior to heating at 48°C for 15 minutes was 3.5 ± 0.1 (mean ± S.E.) units for high-activity samples, and was 3.7 ± 0.1 units for the low-activity group. In a separate experiment in which a different quantity of partially purified enzyme was added to lysates that had already been heated, the recovery of added enzyme was 2.1 ± 0.2 units for high-activity samples and 2.4 ± 0.1 units for the low-activity group. In neither case were the differences between the two groups significant. In addition, lysates from subjects with high enzymatic activity were mixed with equal volumes of lysates from subjects with low activity prior to incubation at 48°C. In each case the rate of inactivation of the mixture was compatible with the independent inactivation of each lysate. These results make it unlikely that the differences in thermostability of COMT in lysates from the three subjects with low and three subjects with high enzyme activity were due to differences in endogenous activators, inhibitors, or COMT degrading enzymes.

Although these results suggested a difference in the thermostability of COMT in erythrocytes of subjects with genetically determined low and high enzyme activity, it remained to be determined whether the results could be confirmed in a larger population. Therefore, as a test of thermostability, COMT activity in lysates from 316 randomly selected adult blood donors was measured before and after the lysates were heated at 48°C for 15 minutes. All assays were performed 1 day after the blood samples were obtained. The data (see Fig. 2) confirm our previous results (*4*, *5*) and show a nonunimodal distribution of erythrocyte COMT activity with a subgroup of subjects with less than 8 units of activity per milliliter of erythrocytes in the unheated samples (Fig. 2A). The subgroup with low COMT activity includes 26.6 percent (84 out of 316) of the population. The percentage of subjects with low COMT activity is similar in males (25 percent, 46 out of 182) and females (28 percent, 38 out of 134). These results may be compared with those in a previous study of 577 subjects in which 23 percent of subjects had an enzyme activity of less than 8 unit/ml (*5*). The distribution of COMT values after heating the lysates at 48°C for 15 minutes (Fig. 2B) also has a nonunimodal distribution with a subgroup with low enzyme activity that includes

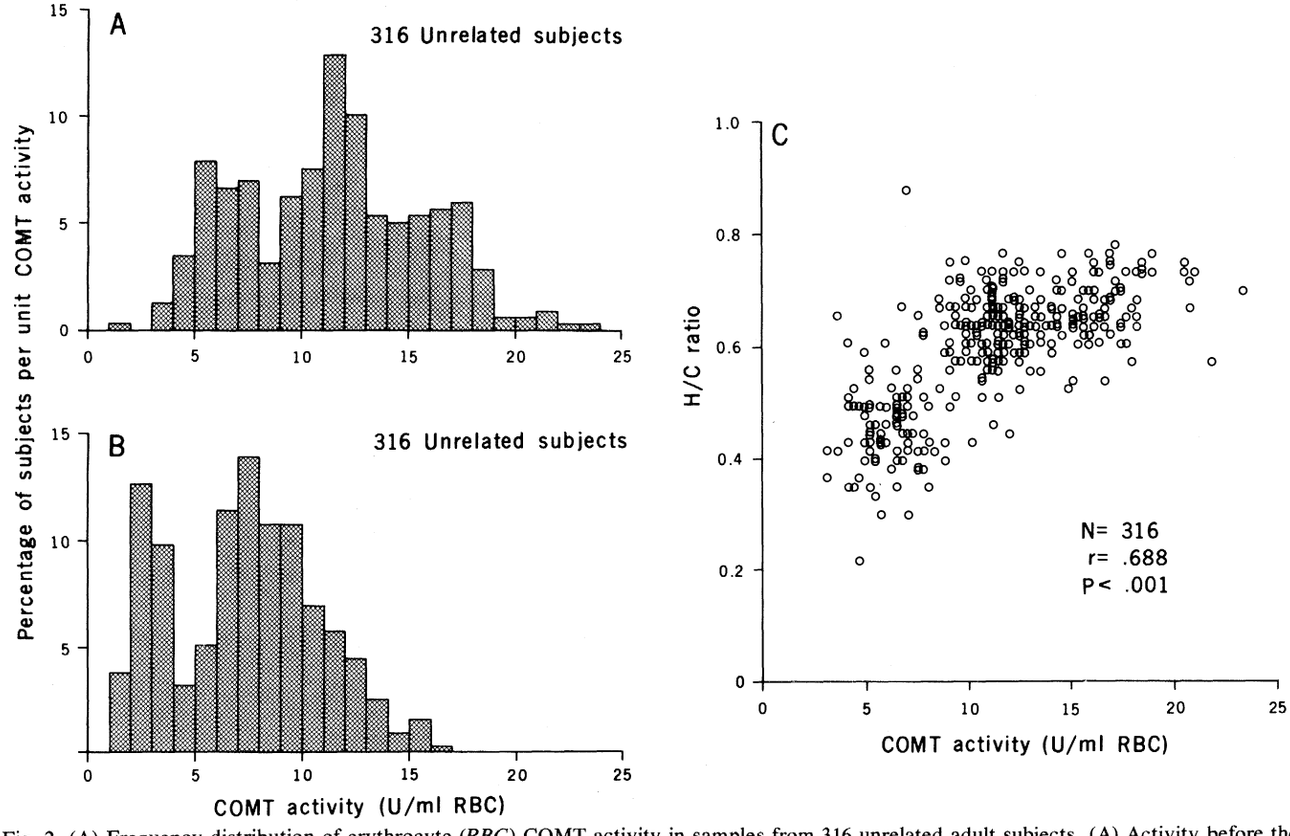


Fig. 2. (A) Frequency distribution of erythrocyte (*RBC*) COMT activity in samples from 316 unrelated adult subjects. (A) Activity before the samples were heated. (B) Activity after the samples were heated at 48°C for 15 minutes. (C) Correlation of COMT activity in unheated erythrocyte lysates with heated/control (*H/C*) ratios (see text for details).

approximately 27.5 percent (87 out of 316) of the population if the separation is made at 4.5 units per milliliter of erythrocytes.

When the ratio of enzyme activity after heating (H) to the activity in controls (C) before heating (H/C ratio) is used as a measure of COMT thermostability, there is a highly significant correlation of H/C ratios with basal erythrocyte COMT activities for these 316 subjects ($r = .688$, $P < .001$; Fig. 2C). Of more importance, Fig. 2C shows that the H/C ratios are lower in subjects with erythrocyte COMT activity of less than 8 unit/ml—that is, subjects homozygous for the allele for low enzyme activity—than in the remainder of the population. The average H/C ratio for the 84 subjects with enzyme activity of less than 8 unit/ml is $0.47 \pm 0.01$ (mean ± S.E.), whereas that for the 232 other subjects studied is $0.65 \pm 0.01$ ($P < .001$). Therefore, these results confirm those obtained in the initial six subjects and are compatible with the conclusion that there is a difference in at least one biochemical property of COMT in the erythrocytes of subjects with genetically different levels of enzyme activity.

It has not been possible to separate subjects heterozygous for the allele for low COMT activity from subjects homozygous for the alternative allele or alleles on the basis of enzyme activity alone (*5*). It would also be difficult to separate these two groups on the basis of the thermostability of COMT, because most of the enzyme activity in erythrocytes of both groups would be the thermostable form and differences in thermostability between these two groups would be small. Therefore, although the inheritance of the trait of low COMT activity might be autosomal codominant rather than autosomal recessive, this question might best be answered with family studies. In addition, family studies will be necessary to verify that the traits of low erythrocyte COMT activity and of thermolability segregate together.

The existence of differences in the thermostability of erythrocyte COMT in lysates from subjects with low and high enzyme activity, levels of activity that are under genetic control (*4, 5, 12*), suggests inherited differences in the structure of the COMT molecule in the human erythrocyte. We propose that the locus responsible for the genetic regulation of human erythrocyte COMT activity be referred to as *COMT*, and that the alleles for "low" and "high" enzyme activity be designated $COMT^{L}$ and $COMT^{H}$, respectively. These designations conform to the recommendations of the Committee on Nomenclature of the Third International Workshop on Human Gene Mapping (*13*). Although the possibility of genetically determined posttranslational modification of COMT cannot be eliminated, the results of our experiments suggest that the locus *COMT* might represent the structural gene for the human enzyme. However, other genetic or environmental factors might participate in the regulation of COMT activity. It remains to be determined whether genetically mediated differences in COMT activity in the erythrocyte, differences which reflect relative COMT activity in the human lung and kidney, are of importance in individual variations in the metabolism of endogenous catecholamines; of catechol drugs such as isoproterenol, α-methyldopa, and L-dopa; and of hormones such as the catecholestrogens (*1, 2, 14*).

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## Octopamine Receptors, Adenosine 3′,5′-Monophosphate, and Neural Control of Firefly Flashing

Abstract. *An adenylate cyclase activated as much as 25-fold by low concentrations of octopamine has been identified in the firefly lantern. The relative potency of octopamine and various other amines in stimulating this enzyme, and effects of antagonists in blocking octopamine activation, correlate well with the known effects of these agents in affecting light production. In addition to suggesting a role for adenosine 3′,5′-monophosphate (or pyrophosphate) in the neural control of firefly flashing, identification of this potent enzyme should facilitate the characterization of phenylethylamine receptors in excitable tissue.*

Octopamine is a naturally occurring phenylethylamine (similar in structure to norepinephrine) found in the tissues of a number of vertebrate and invertebrate species (*1, 2*). In 1973, Nathanson and Greengard (*3*) identified an adenylate cyclase activated specifically by low concentrations of octopamine. The neuronal localization of this enzyme, together with studies of octopamine metabolism (*4*), led to speculation (*1, 3, 5*) that octopamine might function as a neurotransmitter or neuromodulator in the nervous system of invertebrates. Subsequent electrophysiological (*6*) and anatomical (*7*) studies in mollusks, crustaceans, and insects have confirmed the existence of octopaminergic neurons.

Because characterization of hormone-sensitive adenylate cyclases in various tissues has revealed a close similarity between the properties of these enzymes and the known physiological and pharmacological properties of hormone receptors (*8*), efforts have been made to characterize the octopamine receptor by studying octopamine-sensitive adenylate cyclase (*9*). To date, such experiments have been hampered by the lack of a tissue preparation free from other amine receptors. I now report the identification, in the firefly lantern (or "light organ"), of a potent and highly specific octopamine-sensitive adenylate cyclase. The pharmacological characteristics of this enzyme suggest that it may be involved in neural control of lantern flashing.

The timing of firefly light flashes


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