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Cathechol-O-Methyltransferase Activity in Cultured Human Skin Fibroblasts From Controls and Patients With Dystonia Musculorum Deformans

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Fibroblasts provide a source of living cells that can be obtained easily from humans and used to evaluate inherited differences in the activities of enzymes important in neurotransmitter and drug metabolism. Here, we describe biochemical characteristics of catechol-O-methyltransferase (COMT, EC 2.1.1.6) activity in homogenates of cultured human skin fibroblasts. Many properties of the enzyme, including apparent affinity for dihydroxybenzoic acid and S-adenosyl methionine, optimal pH and (Mg^{++}), and inhibition by Ca^{++}, are similar to those reported in lysates of human erythrocytes. Culture and assay conditions have been established for optimal and reproducible measurement of COMT activity in individual fibroblast lines. In 16 control lines, COMT activity ranged from 115 to 263 pmol/min/mg protein with a mean of 181 pmol/min/mg protein. Enzyme activity did not vary with the age or sex of the donor. The COMT activities in fibroblasts from eight patients with dystonia musculorum deformans, an inherited movement disorder of unknown etiology, were not significantly different from controls. Monoamine oxidase (MAO, EC 1.4.3.4) type A activity was measured in 12 lines from patients with dystonia, and values did not differ significantly from age- and sex-matched controls. We conclude that inherited variation in activity of these two catabolic enzymes is not sufficient to explain alterations in monoamine metabolism described in this disorder.

Key words: **catechol-O-methyltransferase, monoamine oxidase, dystonia musculoram deformans, catecholamine metabolism, human fibroblasts, movement disorder, extrapyramidal disorder, cell culture**

INTRODUCTION

A number of properties critical to neural function have been described in cultured human skin fibroblasts, including β-adrenergic-stimulated cyclic AMP accumulation [Haslam and Goldstein, 1974], high-affinity choline uptake [Riker et al, 1981], β-nerve growth factor [Schwartz and Breakefield, 1980]. glutamic acid decarboxylase activity [Gray and

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Dana, 1979], and voltage-dependent sodium channels [Munson et al, 1979], as well as activities of enzymes involved in the catabolism of amine transmitters – monoamine oxidase (MAO) [Groshong et al, 1978; Edelstein et al, 1978], phenol-sulfotransferase [Crooks et al, 1978], and COMT [Groshong et al, 1978; Edelstein et al, 1978]. The present study represents the first detailed characterization of COMT activity in human fibroblasts.

Properties of COMT have been assessed in a number of other tissues [Axelrod and Tomchick, 1958; Axelrod and Cohn, 1971; White and Wu, 1975; Borchardt and Cheng, 1978; Raymond and Weinshilboum, 1975; Huh and Friedhoff, 1979; Axelrod and Vesell, 1970]. This enzyme catalyzes the methylation of endogenous catecholamine transmitters [Axelrod and Tomchick, 1958; Axelrod and Cohn, 1971], catechol estrogens [Ball et al, 1975], and catechol drugs, such as propranolol [Connolly et al, 1972] and L-DOPA [Goodall and Alton, 1972]. Catechol-O-methyltransferase in human brain and liver is predominantly a soluble, monomeric enzyme with a molecular weight of 24,000 daltons and an isoelectric point of 5.5 [White and Wu, 1975]. Extensive measurements of COMT in human erythrocytes have revealed that levels of activity are inherited and that the major determinant of activity is encoded in a single gene locus [Weinshilboum and Raymond, 1977; Winter et al, 1978; Grunhaus et al, 1976; Gershon et al, 1980; Scanlon et al, 1979]. Since the low activity variant is also heat-labile [Scanlon et al, 1979], it is reasonable to expect that this gene codes for the enzyme itself and that structural variations in the amino acid sequence of the enzyme may underlie differences in levels of activity and thermal stability. If this is the case, and if neither isozymic forms (more than one gene locus coding for the enzyme) [Hoffman-Ostenhoff, 1978] nor multiple forms of the enzyme (differences due to posttranslational modification, cellular localization, etc) exist, then it can be assumed that individuals expressing relatively low levels of activity in erythrocytes will have proportionally lower levels throughout their bodies than will other individuals. Activity levels do correlate in a number of tissues from rat and human individuals [Quiram and Weinshilboum, 1976; Weinshilboum, 1978]. However, the lack of correlation between activity levels in human erythrocytes and fibroblasts [Groshong et al, 1978; Giller et al, 1980] and the apparent kinetic and structural differences between soluble and particulate forms of COMT [Huh and Friedhoff, 1979; Axelrod and Vesell, 1970] indicate the need for continued studies on the molecular basis of variations in COMT activity.

We have determined critical conditions of culture, optimal conditions for assay, and kinetic parameters of COMT in human fibroblasts. Activity has been measured in fibroblasts from controls and individuals with dystonia musculorum deformans, an inherited movement disorder in which altered levels of catecholamines and their metabolites have been described in plasma and cerebrospinal fluid (CSF) from patients [Wooten et al, 1973; Ziegler et al, 1976] (Riker et al, unpublished data). Monoamine oxidase activity has also been assessed in dystonic cell lines and compared with values previously obtained for control lines.

MATERIALS AND METHODS

Cell Culture

Fibroblast lines designated as "HF" were established in our laboratory from primary cultures of skin punch biopsies, using a minced explant procedure [Edelstein and Breakefield, 1980]. Biopsies were obtained from the volar forearm or medial thigh above the iliac crest. Other lines (in parentheses) were obtained as established cultures from the follow-

ing sources: American Type Culture Collection, Bethesda, Maryland (Rid Mor, Ro Bel, El San); Institute for Medical Research, Camden, New Jersey (designated as GM); Dr. Samuel Goldstein, McMaster University, Hamilton, Ontario (A1); Dr. Maurice Mahoney, Yale University (82, 86). All lines were from Caucasian individuals and were studied prior to the onset of senescence. Viable frozen stocks of lines were maintained in the vapor phase of liquid nitrogen.

Cells were grown routinely as monolayer cultures in Dulbecco's modified Eagles medium (DMEM, Grand Island Biologicals) supplemented with 5% or 10% fetal calf serum (Flow) at 37°C in a humid atmosphere of 5% CO_2 and 95% air [Edelstein et al, 1978]. Cell stocks were fed at 4–6-day intervals and subcultured at a ratio of 1:5 or 1:7 every 2–3 weeks.

For enzyme assay, cells were plated onto 150-mm tissue culture dishes (Corning), fed at 4–6-day intervals and 1–4 days before harvesting. For COMT, cells were fed with the above medium and harvested 1–2 days after reaching confluency. For MAO, cells were fed with medium containing 5% heat-inactivated serum (56°C, 45 min) and harvested 2–4 days after reaching confluency.

Fibroblast Homogenates

Cultures were harvested after rinsing monolayers three times with isotonic phosphate-buffered saline and scraping cells into a small volume of 0.1 M (for COMT assays) or 0.01 M (for MAO assays) potassium phosphate buffer, pH 7.4, as described [Edelstein et al, 1978]. Cell suspensions containing 1–5 mg protein per ml were frozen immediately on dry ice and stored at −60°C. Prior to assay, suspensions were thawed, held on ice, and sonicated three times for 10 sec using a microprobe (Biosonik IV, Bronwill).

COMT Assay

Enzyme activity was determined by a modification of the method of Raymond and Weinshilboum [1975]. The following procedure was used unless otherwise indicated. Thirty-microliter aliquots of homogenates were placed in 15-ml glass screw-cap tubes. Enzyme reactions were initiated by the addition of 20 μl of a mixture containing the following reagents (final concentration in 50 μl indicated): Tris-HCl pH 7.0, 0.1 M; $MgCl_2$, 1 mM; S-adenosyl-L-[$Me^{14}C$]- methionine (specific activity 46.6 mCi/mmol, New England Nuclear, toluene extracted), 20 μM; nonradioactive S-adenosyl-L-methionine HCl, 0.1 mm; dihydroxybenzoic acid, 2.5 mM; dithiothreitol, 4 mM; and EGTA, 1 mM. (For optimal assay, the reaction should be run at pH 7.5 using 0.5 mM dihydroxybenzoic acid.) Reactions were incubated for 30 min at 37°C in a shaking water bath, and terminated by addition of 200 μl of 1 N HCl and 12 ml of toluene. Capped tubes were shaken horizontally for 5 min at room temperature and then centrifuged at 500g for 10 min. Four ml of the organic phase was added to vials containing 7 ml scintillation fluid (4% (v/v) Liquifluor (New England Nuclear) in toluene). An aliquot of the cocktail was counted in the same scintillation fluid containing 33% (v/v) Triton X-100 (New England Nuclear).

All results were corrected for the extraction of 4-hydroxy-3-methoxybenzoic acid into the organic phase (54%). Authentic labeled 4-hydroxy-3-methoxybenzoic acid was isolated following thin-layer chromatography of the methylated reaction products produced by fibroblast homogenates. Thin-layer chromatography was performed as described [Raymond and Weinshilboum, 1975].

Assay blanks consisted of the complete reaction mixture minus dihydroxybenzoic acid. The radioactivity in these blanks was approximately twice as high as either zero time

blanks or blanks lacking homogenate, suggesting that other compounds are also methylated and extracted. Activity was completely inhibited by 1 mM S-tubercidinyl-methionine (a COMT inhibitor [Coward et al, 1974] kindly provided by Dr. James Coward). Reactions were linear with time (up to 30 min) and protein (up to 250 μg). A standard rat liver enzyme preparation [Coyle and Henry, 1973] was used as a control in assays; COMT activity in this preparation varied $\leqslant$10% from assay to assay. A mixture of this preparation with a control fibroblast homogenates yielded COMT activity that was 10–20% less than the sum of activities measured separately.

For routine determinations of COMT, each line was assayed in triplicate using homogenates from two or more separate subcultures. Protein was determined by the method of Lowry et al [1951], using bovine serum albumin as a standard.

To establish the apparent affinity (K_m) of COMT for S-adenoxyl methionine and dihydroxybenzoic acid reactions were carried out at six concentrations over a range of 3–48 μM and 50 to 250 μM, respectively.

MAO Assay

Enzyme activity was determined by toluene extraction of the deaminated products of ^{3}H-tryptamine as described [Edelstein et al, 1978]. The concentration of ^{3}H-tryptamine used in this assay, 40 μM, is saturating for the enzyme, and MAO activity of the A type is measured predominantly [Breakefield et al, 1979]. Reactions were linear with time and protein. Values for each line were determined at two protein concentrations in triplicate using homogenates from 2–6 separate subcultures.

Inferential Statistics

To compare frequency distributions of enzyme activity for control and dystonic fibroblasts, we applied the Kolmogorov-Smirnov test [Siegel, 1956]. This two-sample, nonparametric test is sensitive to differences in the location, dispersion, and skewness of sample distributions and is applicable to the comparison of nonnormal and skewed distributions.

RESULTS

Biochemical Characterization of COMT

Several properties of COMT in fibroblast homogenates were determined and compared with measurements reported in erythrocyte lysates by Weinshilboum and co-workers [Raymond and Weinshilboum, 1975; Scanlon et al, 1979] (Table I). As for erythrocytes, when measuring COMT in fibroblasts, it is critical to remove free Ca^{++}. In fibroblasts, this was done by including EGTA in the reaction mixture. No further increase in activity was observed when homogenates were pretreated with Chelex-100 resin which is used with erythrocyte lysates [Raymond and Weinshilboum, 1975]. The inclusion of dithiothreitol in the reaction mixture was necessary for maximal activity of COMT in fibroblast homogenates and resulted in a 165% increase in activity, as compared to a 15% increase seen in erythrocyte lysates. The pH optimum for COMT in fibroblast homogenates was broad, with an optimum between pH 7.4 to 7.6 (measured for line GM2037 over a range of pH 7.0–8.4 in 0.1 M Tris-HCl buffer), as compared to pH 7.7–7.8 in erythrocyte lysates [ibid]. The optimal Mg^{++} concentration for both preparations was 1 mM (Fig. 1, Table I).

The apparent substrate affinity (K_m) of COMT in fibroblast homogenates for S-adenosyl-methionine and dihydroxybenzoic acid was determined by Lineweaver-Burk

TABLE I. Comparison of COMT in Fibroblasts and Erythrocytes

Property[a]	Fibroblasts	Erythrocytes
Increase in absence of Ca^{++}	56%	87%[b]
Increase in presence of DTT	165%	15%[b]
Optimal pH	7.4–7.6	7.6–7.8[b]
Optimal (Mg^{++})	1 mM	1 mM[b]
K_m SAM	13 mM[c]	7 mM[b,d]
K_m DHBA	40 mM[c]	70 mM[b,d]
Substrate inhibition at 2 mM DHBA	16%	4%[b]

[a]Abbreviations: DHBA, dihydroxybenzoic acid; SAM, S-adenoyl-methionine; DTT, dithiothreitol.

[b]From Raymond and Weinshilboum [1975]. In another publication, Scanlon et al [1979], using similar procedures, apparent K_m values of 3.5 mM and 15 mM were obtained for SAM and DHBA, respectively.

[c]Mean values are given from the results of 2–3 experiments using homogenates from two different lines.

[d]In our laboratory, we observed apparent K_m values of 15 mM for SAM and 93 mM for DHBA; substrate inhibition at 2 mM DHBA was 18%.

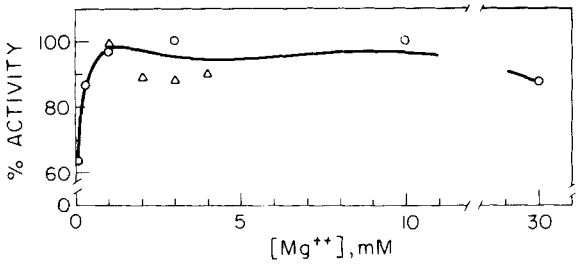

Fig. 1. COMT activity in fibroblasts as a function of Mg^{++} concentration. The final concentration of Mg^{++} in the reaction mixture was varied from 0.2 mM to 30 mM. Data are shown from two experiments using homogenates from line 64 (○) and GM316 (△).

analysis [Lineweaver and Burk, 1934]. The apparent K_m for S-adenosyl-methionine measured in homogenates of lines GM23 and 82 was 15 μM and 11 μM, respectively, about 3 to 6-fold higher than that reported for erythrocyte lysates [Raymond and Weinshilboum, 1975; Scanlon et al, 1979] and purified enzyme from human brain and liver [White and Wu, 1975], but similar to that observed by us in erythrocyte lysates (Table I). The K_m for dihydroxybenzoic acid was 38 μM in one subculture of line GM302, and 33 μM and 49 μM in two separate subcultures of line GM1651. This apparent substrate affinity is within the range of that observed in erythrocyte lysates (Table I) and in human brain and liver [White and Wu, 1975]. All three fibroblast lines tested showed substrate inhibition at concentrations of dihydroxybenozic acid greater than 1 mM (Fig. 2) as did erythrocyte lysates in our hands (Table I).

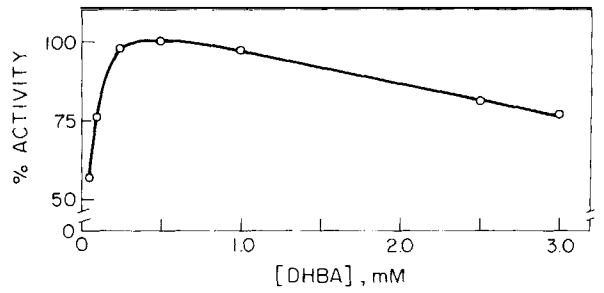

Fig. 2. COMT activity in fibroblasts as a function of the concentration of dihydroxybenzoic acid. The final concentration of this substrate in the reaction mixture was varied from 0.1 mM to 3 mM. The homogenate was prepared from line GM1651; 100% activity was 200 pmol/min/mg protein. Similar results were observed in separate experiments with lines GM1651 and GM302.

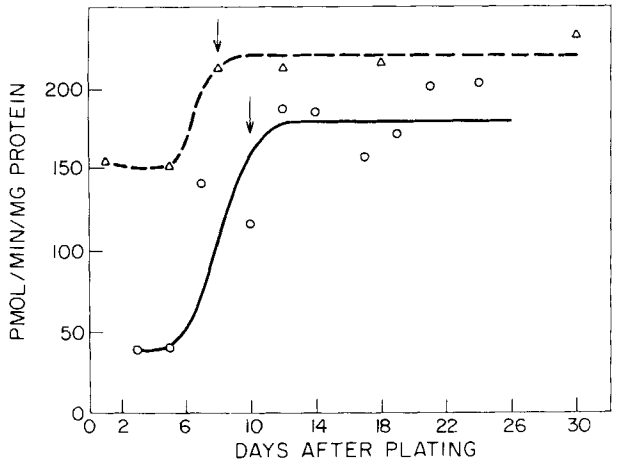

Fig. 3. COMT activity as a function of cell density. Lines El San (△) and HF27 (○) were plated at a density of 2×10^3 cells/cm on day 0. Cultures were fed every 4 days and parallel plates were harvested at intervals indicated. COMT activity was measured in homogenates by the standard procedure. Arrows indicate the times at which the cultures became confluent.

Whereas enzyme activity in erythrocyte lysates is about 90% soluble (not pelleted by centrifugation at 100,000g for 1 h), activity in fibroblast homogenates is only about 50% soluble (by this criterion).

Effect of Culture Conditions of COMT Activity

Control lines were grown under different conditions to establish which variables of culture would affect levels of COMT activity. The following variables were tested: Frequency of feeding prior to harvesting, presence of serum, type of medium, stage of growth, and successive subcultures.

Two lines (HF8 and GM1651) were subcultured in parallel onto a series of dishes. After monolayers reached 100% confluency, cultures were fed DMEM with 10% fetal calf serum at the following intervals: After 2 days; after 2 and 6 days; and after 2, 4, 6, and 7 days. All dishes were harvested 10 days after reaching confluency. Catechol-O-methyltransferase activity did not vary as a function of these feeding schedules (data not shown). Parallel cultures fed DMEM with 10% heat-inactivated (55°C for 45 min) fetal calf serum, or MCDB104 medium without serum [McKeehan et al, 1977] 5 and 6 days after reaching confluency had similar levels of COMT activity.

Two lines (El San and HF27) were subcultured in parallel onto a series of dishes at a density of about 2×10^3 cells/cm^2. Cultures were harvested periodically over a 30-day interval. Levels of activity increased 30% in El San and 80% in HF27 as cultures progressed from the logarithmic to the stationary phase (100% confluency) of growth (Fig. 3). After monolayers reached confluency, the level of COMT activity remained essentially constant for up to 2 weeks.

Catechol-O-methyltransferase activity was stable for individual lines (eg, GM1651 and HF8) over successive subcultures (10 and 12, respectively) when monolayers were harvested 1–2 days after reaching confluency (data not shown). Line GM1651 was nearing the end of its proliferative lifespan in culture at the last subculture tested.

COMT Activity in Control and Dystonic Fibroblasts

Using standardized procedures for cell growth and enzyme assay, COMT activity was measured in 16 fibroblast lines from control individuals ranging in age from 1 to 31 yrs (Table II). The mean activity level was 181 pmol/min/mg protein, with values ranging from 115 to 263. There was no apparent variation in activity as a function of age or sex of donor. A plot of the frequency distribution of COMT activity in control lines revealed a unimodal distribution with a slight positive skew. Fibroblasts from one set of monozygotic twins had essentially identical levels of COMT activity. Similar levels of activity were also observed in pure populations of papillary and reticular fibroblasts [Harper and Grove, 1979] from the same control female donor (in collaboration with Dr. Robert A. Harper, Temple University School of Medicine, Philadelphia; data not shown).

Activity levels in fibroblasts from six patients with dystonia musculorum deformans (both recessively and dominantly inherited forms, ages 4–31 yrs) fell entirely within the control range. The mean for the dystonic population was 178 pmol/min/mg protein with values ranging from 120 to 238. A two-sample Kolmogorov-Smirnov test (one-tailed) revealed no significant difference between control and dystonic distributions of COMT activity ($P \gg 0.05$; $D = 0.15$; $\chi^2 = 0.514$). Similarly, COMT activity in relatives of dystonic patients fell within the normal range.

MAO Activity in Fibroblasts From Patients With Dystonia

In lines from 12 individuals (13–38 yrs of age) with diagnosed dystonia, MAO activity ranged from 1.2 to 43 pmol/min/mg protein (Table III). A plot of the frequency of MAO activity in this population revealed a unimodal sampling distribution with a strong positive skew. All values fell within the range for control fibroblasts. In lines from 57 Caucasian control individuals (<1 to 72 yrs), values range from 0.7 to 114 pmol/min/mg protein [Edelstein et al, 1978; Costa et al, 1980, unpublsihed data]. A two-sample Kolmogorov-Smirnov test (one-tailed) of MAO activity in dystonic probands ($N = 12$; 13–38 yrs) and age-matched controls ($N = 36$; 12–38 yrs) revealed no significant difference in distribution ($P > 0.1$; $D = 0.333$; $\chi^2 = 3.99$).

TABLE II. COMT Activity in Dystonic and Control Fibroblasts

Line	Type[a]	Age (yrs)	Sex	Activity (pmol/min/mg protein)
Dystonics				
GM2255	R	4	F	191.2 ± 31.7 (3)[b]
HF41	–	13	F	164.1 ± 14.9 (3)
GM2306	R	13	M	168.1 ± 12.0 (3)
GM2304	D	17	F	228.6, 248.1
HF 60	D	19	F	104.2, 135.6
GM2551	R	31	F	185.1, 173.3
Relatives of dystonics[c]				
HF59		16	F	153.8 ± 11.2 (3)
HF61		43	F	128.7, 130.4
HF45		26	M	125.5, 131.1
HF47		28	F	108.1, 113.8
HF39		54	M	160.6 ± 26.7 (3)
HF40		53	F	174.8 ± 10.2 (3)
Controls				
GM302		1	M	170.7, 178.1
GM409		7	M	133.3, 180.2
GM499		8	M	250.1, 275.6
GM500		10	M	180.0 ± 8.2 (3)
GM323		11	M	111.4, 118.2
GM316[d]		12	M	151.8 ± 28.4 (3)
GM2037[d]		13	M	177.7 ± 7.7 (3)
GM1651		13	F	160.1 ± 6.8 (3)
GM1661		14	F	170.5 ± 27.1 (3)
Robel		14	M	148.8, 184.9
86		15	F	170.4 ± 9.3 (3)
Rid Mor		15	M	138.7, 183.4
Al[d]		15	M	177.6, 211.0
GM498[d]		30	M	200.0 ± 7.7 (3)
HF23[e]		31	F	222.3, 230.2
HF22[e]		31	F	219.6, 243.8

[a]Refers to mode of inheritance: D, dominant; R, recessive; –, indeterminant.
[b]Expressed as mean ± SEM (N), where N = number of separate subcultures assayed. When N = 2, both values are listed. Values were measured using 2.5 mM dihydroxybenzoic acid and thus represent about 76% of the activity at the optimal substrate concentration, 0.5 mM.
[c]Lines were obtained from: HF59, sister of DHF60; HF61, mother of HF60; HF45 and HF47, first cousins of HF48; HF39, father of HF41; HF40, mother of HF41.
[d]These lines were previously reported as age and sex-matched controls [Giller et al, 1980].
[e]Monozygotic twins.

DISCUSSION

There are several advantages in using fibroblasts as compared to erythrocytes to assess COMT activity in the human population. First, activity can be compared in cells grown under controlled environmental conditions, thus avoiding many physiological variables encountered in vivo. Second, COMT can be measured in homogenates prepared from actively metabolizing, nucleated cells. Third, other enzymes involved in the degradation of catecholamines can be assessed concurrently. Fourth, these cells can be frozen away viably and studied by a number of laboratories. The main limitation to the use of fibroblasts is the effort involved in the cell culture.

TABLE III. MAO Activity in Dystonic Fibroblasts

Line	Type[a]	Age (yrs)	Sex	Activity[b] (pmol/min/mg protein)
HF41	–	13	F	43.3 ± 5.0 (3)
GM2306	R	13	M	1.2 ± 0.2 (3)
GM2304	D	17	F	0.5 ± 0.1 (3)
GM3210	–	21	M	5.5, 6.8
GM3218	–	25	M	6.7, 8.1
GM3213	–	26	F	4.1, 6.1
GM3208	–	29	M	29.9, 32.0
HF49	D	30	F	23.4 ± 5.5 (3)
GM3211	–	30	M	23.1, 27.8
GM2551	R	31	F	3.0 ± 0.7 (4)
HF48	D	32	F	3.0, 4.3
HF50	D	38	M	22.0 ± 6.5 (3)

[a]Refers to mode of inheritance: D, dominant; R, recessive; –, indeterminate.
[b]Expressed as mean ± SEM (N), where N = number of separate subcultures assayed. When N = 2, both values are listed.

Fibroblasts can be used to evaluate the inherited ability of an individual to metabolize biogenic amines in vivo. Two criteria are important to this evaluation. First, the culture conditions that affect the property need to be standardized to minimize this as a source of variation in activity. Second, the number of forms of the enzyme (isozymes) that exist in different human tissues needs to be established. If there is only one form of the enzyme, and if inherited variations in the structure of the enzyme are the primary determinants of activity, then by measuring activity in one cell type, one can infer the functional activity of the enzyme in other tissues in vivo. If more than one form of an enzyme exists, one needs to assess all forms and to elucidate their domains of activity in vivo.

Biochemical properties of COMT measured in fibroblasts are similar to those in erythrocytes [Raymond and Weinshilboum, 1975; Scanlon et al, 1979] with respect to inhibition by Ca^{++}, optima for pH and $[Mg^{++}]$, and substrate affinities for S-adenosylmethionine and dihydroxybenzoic acid. Enzyme activity in fibroblast homogenates is more markedly increased by dithiothreitol than in erythrocyte lysates, suggesting the presence of higher levels of sulfhydryl oxidizing agents in fibroblasts. Furthermore, when activity in erythrocytes is expressed as pmol/min/mg protein (instead of pmol/min/ml packed red cells), erythrocytes have about 1/400 of the activity of fibroblasts. Although in erythrocytes almost all the activity is soluble, in fibroblasts about 50% of the activity appears to be associated with membranes. Together, these findings suggest that the form of COMT being expressed in fibroblasts may not be identical to that in erythrocytes, and/or that other cellular components that differ between these two cell types may modulate expression of activity.

Culture conditions are described here that allow reproducible measurement of COMT activity in individual fibroblast lines from subculture to subculture. As with MAO [Edelstein et al, 1978], COMT activity is lower in fibroblasts during the logarithmic than the stationary phase of growth. By harvesting cultures after confluency is reached, one can avoid the effect of cell division on enzyme activity. In contrast to MAO [Edelstein et al, 1978], COMT activity was not found to be sensitive to variations in feeding schedule, type of medium, or presence of serum. The finding that levels of COMT activity are similar in

fibroblasts from monozygotic twins supports the assumption that levels are under genetic control. Furthermore, the similarity of activity levels in pure papillary and reticular fibroblasts from the same individual indicates that this measurement is not confounded by varying proportions of these two cell types in fibroblast lines.

Fibroblasts can be used to evaluate inherited variations in COMT and MAO (Type A) activity within the human population, as well as the role of these variations in neurologic and psychiatric diseases. In prior studies, we have observed normal levels of COMT activity in fibroblasts from patients with the Gilles de la Tourette syndrome, autism [Giller et al, 1980], and the Lesch-Nyhan syndrome [Roth et al, 1976]. Furthermore, normal levels of MAO have been found in patients with bipolar depressive illness [Breakefield et al, 1980], Huntington's disease, familial dysautonomia [Edelstein et al, 1979], autism, Gilles de la Tourette syndrome [Giller et al, 1980] and the Lesch-Nyhan syndrome [Edelstein et al, 1978]. In the latter two diseases, however, levels of MAO activity measured in fibroblasts appear to correlate with clinical severity.

Here, we have examined activities of COMT and MAO (Type A) in fibroblasts from patients with dystonia musculorum deformans. The primary etiology of this movement disorder, characterized by tonic spasms and clonic contractions of skeletal muscles, is not known, but studies of neurotransmitter metabolism in vivo have suggested possible alterations in biogenic amine turnover. Two forms of the disease have been identified based on the mode of inheritance in pedigrees. The dominant form has a later age of onset and a larger number of non-Jewish patients than the recessive form. The dominant form is also characterized by very high levels of norepinephrine (NE) and dopamine β-hydroxylase in the plasma [Wooten et al, 1973; Ziegler et al, 1976], and increased NE in CSF [Riker et al, unpublished data] of patients. Many of both types of patients have low levels of the dopamine metabolite, homovanillic acid (HVA), and the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in plasma and CSF [Riker et al, unpublished data]. The results reported here on MAO-A and COMT in dystonic fibroblasts suggest that inherited variations in the activity of these catabolic enzymes cannot explain either the increased steady-state levels of NE in plasma and CSF in dominant dystonia, nor the decreased steady-state levels of HVA and 5-HIAA in the CSF in both dominant and recessive dystonia. Together, these findings direct attention toward possible deficiencies in synthesis, storage, release and reuptake of biogenic amines in this disease.

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