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Variation in hepatic membrane-bound catechol-*O*-methyltransferase activity in Fischer and Wistar–Furth strains of rat

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Catechol-*O*-methyltransferase (COMT) is one of the major enzymes involved in the inactivation of the catecholamine neurotransmitters dopamine, norepinephrine and epinephrine [1, 2]. Two distinct forms of COMT have been identified [3–7], a soluble and a membrane-bound species, possessing similar biochemical and kinetic properties [8, 9] although having different molecular weights on sodium dodecyl sulfate (SDS) gels of 23 and 26 kD respectively [10]. The major biochemical difference between the two forms of COMT is that the catecholamine substrates possess a considerably higher affinity for the membrane-bound form of the transferase by a factor of approximately 100 [2, 9, 11]. For example, the K_m value for dopamine binding to the human membrane-bound COMT is approximately 3 μM, whereas this value is 280 μM with the soluble enzyme species [2]. Despite this difference, the soluble enzyme has been regarded, in the past, as the predominant form of COMT responsible for the methylation of catecholamines. In contrast, prior studies [2] have revealed that membrane-bound COMT in human brain has a considerably greater capacity to degrade both dopamine and norepinephrine when these neurotransmitters are present at physiologically relevant concentrations of 10^{-5} M or less. These data suggest that membrane-bound COMT may, in fact, be the predominant form of the transferase responsible for methylation of the catecholamines dopamine and norepinephrine, at least, in human brain.

The soluble form of COMT has been reported to be regulated genetically in that two distinct allelic forms possessing different activities have been shown to exist in the human population [12]. Similarly, genetic differences for the soluble form of COMT have been demonstrated in various strains of rats [13–15]. In this regard, it was observed that the Wistar–Furth rat strain possesses almost twice the soluble COMT activity as that of the Fischer strain.

Given the fact that the membrane-bound form of COMT may be the major form of the transferase in human brain, it would be of interest to determine whether this form of COMT is also regulated genetically. Accordingly, in this paper we initially attempted to establish whether differences in activity of membrane-bound COMT in livers from Wistar–Furth and Fischer strains of rats are comparable to those observed with the soluble enzyme. To determine whether membrane-bound COMT is biochemically similar in these strains of rats, the kinetic constants for dopamine and the methyl donor, *S*-adenosyl methionine (SAM), were determined.

Materials and Methods

Livers from four male Wistar–Furth and four male Fischer rats were removed and homogenized separately with a polytron in 0.05 M potassium phosphate buffer, pH 7.4. The homogenates were centrifuged at 10,000 *g* for 10 min, and the resulting supernatant solution was recentrifuged at 100,000 *g* for 60 min to obtain the microsomal pellets. The microsomes were washed once in phosphate buffer, and the final microsomal fraction was resuspended in buffer and assayed immediately for membrane-bound COMT activity.

The activity of membrane-bound COMT was measured as previously described using [^{3}H]dopamine as the labeled substrate [2]. In brief, reaction mixtures containing 2.5 mM magnesium chloride, 1 mM pargyline (monoamine oxidase inhibitor), 1 mM SAM and 4 μM [^{3}H]dopamine (approx. 150,000 cpm/nmol) were incubated in a total volume of 0.3 mL of 50 mM potassium phosphate buffer, pH 7.4, for 40 min at 37°. To ensure that only membrane-bound COMT activity was measured in our microsomal preparation, a concentration of dopamine that was near its K_m value and approximately 100 times less than the K_m value for the soluble enzyme was chosen. The reactions were stopped with the addition of 0.6 mL of 0.5 M potassium borate, pH 10.0. A mixture (3 mL) of toluene:isoamyl alcohol (3:2, v/v) was added to the solutions which were vortexed for approximately 15 sec. After centrifugation, 1-mL aliquots of the organic phase were removed and assayed for radioactivity by liquid scintillation spectrometry. Reaction velocity was linear with time and protein concentration throughout the duration of the assay. K_m and V_{max} values for dopamine and SAM were determined by linear regression analysis from double-reciprocal plots.

3,4-Dihydroxyphenyl[7-^{3}H]ethylamine ([^{3}H]dopamine: 40 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Dopamine·HCl, *S*-adenosyl-L-methionine (chloride form, 85% pure), pargyline·HCl, and dithiothreitol were obtained from the Sigma Chemical Co. (St. Louis, MO). All other reagents were the highest commercial grades available.

Results and Discussion

The average activity of liver membrane-bound COMT in two separate experiments from Fischer and Wistar–Furth rats is indicated in Table 1. As demonstrated, the livers from Fischer rats contain approximately 60% of the membrane-bound COMT activity as that observed in the Wistar–

Table 1. Activity of membrane-bound COMT in livers of Fischer and Wistar–Furth rats

Expt. No.	Specific activity (pmol/min/mg protein) Fischer	Wistar–Furth	Ratio F/W-F
1	68.1 ± 4.5	117.4 ± 9.5	0.58
2	56.4 ± 4.6	89.4 ± 5.5	0.63

The values reported for specific activity are the means ± SE of four separate livers from each strain of rat for each experiment. The procedure used is described in Materials and Methods.

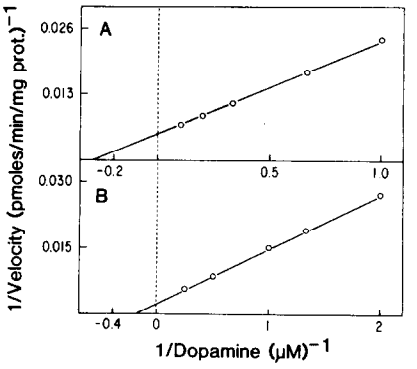

Fig. 1. Lineweaver–Burk plot of liver membrane-bound COMT from Fischer (A) and Wistar–Furth (B) strains of rat using various concentrations of dopamine. Reaction mixtures containing 2.5 mM MgCl, 1 mM SAM, liver microsomes (45–50 μg) and various concentrations of [^{3}H]dopamine in a total volume of 0.3 mL of 50 mM potassium phosphate buffer, pH 7.4, were incubated at 37° for 30 min. Values are the average of reactions performed in duplicate.

Furth animals as measured with 4 μM dopamine. These results are strikingly similar to that observed for the soluble form of COMT in livers from these strains of rats [14].

To determine whether the two species of membrane-bound COMT from the Fischer and Wistar–Furth animals possess similar biochemical characteristics, the K_m and V_{max} values were determined from double-reciprocal plots as illustrated in Figs. 1 (A and B) and 2 (A and B). The average K_m values from three sepearate experiments, indicated in Table 2, reveal that the K_m values for both dopamine and SAM were approximately 27 and 25% lower for membrane-bound COMT from the Fischer rat as compared to that from the Wistar–Furth strain. However, only the difference in the K_m value for dopamine reached statistical significance ($P < 0.05$). The K_m values for dopamine are similar to those reported earlier for rat membrane-bound COMT and demonstrate that the O-methylating activities measured were not caused by contamination of the membrane preparations with the soluble form of COMT which possesses a K_m value for dopamine of approximately 300 times greater [6].

The data reported in Table 2 also demonstrate that the difference in the V_{max} values between the two strains was approximately 55%, with the Fischer strain being lower. This difference is consistent with the results shown in Table 1. The disparity in the K_m and V_{max} values between the two species of membrane-bound COMT suggests that the structures of these transferases in the two strains of rats may be different, although it does not rule out the possibility that the variations observed were simply caused by differences in the composition of the isolated membranes which impart unique functional properties and activities to the two enzymes.

The differences in the activity of membrane-bound COMT observed in this study are similar to those seen previously with the soluble form of the transferase in the

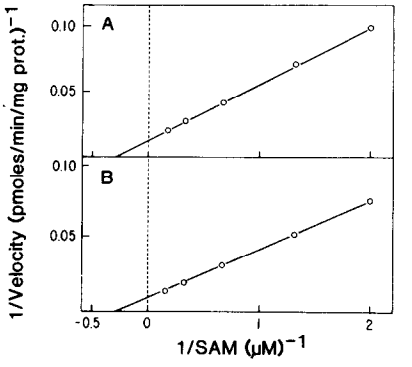

Fig. 2. Lineweaver–Burk plot of liver membrane-bound COMT from Fischer (A) and Wistar-Furth (B) strains of rat using various concentrations of SAM. Reaction mixtures containing 2.5 mM $MgCl_2$, 10 μM [^{3}H]dopamine, liver microsomes (45–50 μg) and various concentrations of SAM in a total volume of 0.3 mL of 50 mM potassium phosphate buffer, pH 7.4, were incubated at 37° for 30 min. Values are the average of reactions performed in duplicate.

Table 2. Kinetic constants for membrane-bound COMT in Fischer and Wistar–Furth rats

Rat strain	K_m (μM) Dopamine	SAM	V_{max} (pmol/min/mg protein)
Fischer	3.05 ± 0.24	2.83 ± 0.43	157 ± 27
Wistar–Furth	4.19 ± 0.27*	3.82 ± 0.25	352 ± 16

Kinetic constants presented are the means ± SE determined from three separated Lineweaver–Burk plots. The procedure is described in Materials and Methods.

* Significantly different from the value for Fischer rats, $P < 0.05$.

two species of rat [14] even though the membrane-bound species represents a small percentage of the total COMT activity in rat liver [6]. These data imply that factors regulating the activity of membrane-bound and soluble COMT may be similar in both strains of rat. In this regard, it is possible that the membrane-bound and soluble forms of COMT are derived from the same gene and only result from different post-translational processes. In support of the hypothesis that both forms of COMT are derived from a single gene is the fact that the biochemical behavior (Mg^{2+} requirement, Ca^{2+} inhibition, and kinetic mechanism) of the two isoenzymes is very similar [16, 17] and that antibodies raised against soluble COMT can cross-react with the membrane-bound species [10, 18, 19]. The 3 kD difference in the molecular weight between the two species of COMT may be attributed to a hydrophobic peptide or glycosylated tail connected to the soluble form of COMT which is required for the attachment of the membrane-bound species to the membranes. Assuming this is true, then the higher affinity of the catecholamine substrates for the membrane-bound COMT may be caused either by the influence of the tail on the tertiary structure of the enzyme or by the lipid environment within the membranes. Ultimately, cloning the cDNA for the soluble and membrane-bound forms of COMT will establish the molecular structure of the two enzymes.

Although the relative differences in the activities of membrane-bound and soluble COMT activities observed in the two strains of rats are consistent with a single gene coding for both enzyme forms, it does not rule out the possibility that two separate genes exist which are simultaneously regulated by a common process. Studies are currently underway to determine the genetic structure and regulation of the soluble and membrane-bound forms of COMT.

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JEROME A. ROTH*†
MARK H. GROSSMAN‡
MAUREEN ADOLF*

*Department of Pharmacology and Therapeutics
State University of New York at Buffalo
School of Medicine and Biomedical Sciences
Buffalo, NY 14214; and
‡Department of Pediatrics
Temple University Medical School
Philadelphia, PA 19133
U.S.A.

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† Send reprint requests to: Dr. Jerome A. Roth, Department of Pharmacology and Therapeutics, SUNY at Buffalo, 102 Farber Hall, Buffalo, NY 14214.