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PRELIMINARY COMMUNICATIONS

PRESENCE OF MEMBRANE-BOUND CATECHOL-O-METHYLTRANSFERASE IN HUMAN BRAIN

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Numerous papers over the past 15 years have demonstrated the existence of a membrane-bound form of the enzyme catechol-O-methyltransferase (COMT) in a variety of animal tissues. The relative amount of this microsomally bound enzyme varies both with the animal species and the organ examined. In mouse liver, for example, this form of the transferase represents almost 70 percent of the total enzymatic activity whereas in rat brain and liver this molecular species accounts for less than 5 percent of the total COMT activity [1,2]. A recent abstract by Sladek and Weinshilboum [3] indicates that human lymphocytes contain approximately 50 percent of their total COMT activity as the membrane-bound enzyme.

Several studies have reported that the molecular and kinetic properties of membrane-bound COMT when solubilized closely resemble those of the soluble transferase. Tong and D'Iorio [4] and Borchardt and Cheng [2] reported that the K_m values for S-adenosyl-L-methionine and several acceptor substrates were similar for the two enzyme species and that the molecular weights of the two enzymes were almost identical. In addition, Borchardt and coworkers [2,5] have also demonstrated that antibodies to the soluble COMT cross-reacted, at least partially, with the purified membrane-bound transferase. Nevertheless, it remains unclear as to whether the two enzymes actually represent distinct protein species with differing intracellular sites or whether the membrane-bound enzyme simply results from non-specific binding of the soluble transferase to the microsomal membranes. In this regard, when bound to the microsomal membrane the properties of COMT are clearly distinguishable from the soluble enzyme. In general, the affinities of the catechol substrates for the membrane-bound enzyme are at least one order of magnitude greater than the affinities of the soluble transferase [4,6,7]. This suggests that the membrane components surrounding COMT in the microsomes play an important role in regulating its substrate specificity and enzymatic activity.

Surprisingly, little is known about the properties of the membrane-bound COMT of human brain. White and Wu [8] have purified and characterized the soluble transferase of human brain but no reference was made as to the presence of the membrane-bound species in this tissue. As described in this paper, we have found that over 50 percent of the COMT activity

in human brain resides in the microsomal fraction and that the specific activity of this enzyme is approximately ten times greater than the soluble transferase.

METHODS

Frontal lobes of human brain were obtained at autopsy usually within 12 hr after death. Brain tissue was homogenized in a Waring Blender for three 15-sec periods followed by homogenization in a Teflon-glass homogenizer. The homogenate was centrifuged twice at 700 g, each for 10 min, to remove large unbroken cell fragments and nuclei. The resulting supernatant solution was centrifuged at 10,000 g for 20 min and then at 100,000 g for 60 min to isolate the microsomal fraction.

Catechol-O-methyltransferase activity was assayed by a modification of the method of Wrenn et al. [9]. In brief, 500 µl of a microsomal or soluble enzyme suspension in potassium phosphate buffer (pH 7.4) was incubated in the presence of 100 µM S-adenosyl-L-methionine, 2.5 mM $MgCl_2$, 1.0 mM pargyline, and various concentrations of ^{3}H-labeled norepinephrine (NE) or dopamine (DA) for 30 min at 37°. The reactions were terminated by the addition of 1 ml of 0.5 M potassium borate (pH 10.0) solution and the radioactive 3-O-methylated derivative was extracted into 5 ml of a toluene/isoamyl alcohol (3:2) mixture. A 1-ml aliquot of the organic phase was removed and assayed for radioactivity by liquid scintillation spectrometry. All reactions were linear both with time and enzyme concentrations. Protein concentrations were determined by the method of Lowry et al. [10].

RESULTS AND DISCUSSION

The specific activities for O-methylation of NE and DA by COMT in microsomal and soluble fractions of human brain tissue are presented in Table 1.

Table 1. Specific activities for O-methylation of norepinephrine and dopamine by human brain catechol-O-methyltransferase*

Substrate	Concn (µM)	Product formed	
		Membrane bound	Soluble
Norepinephrine	2	68.9 ± 2.9	7.8 ± 1.1
Dopamine	2	265 ± 45	12 ± 2
Dopamine	10	505 ± 157	27 ± 8

* Product formed is expressed as pmoles/mg protein for 30 min (mean ± S.E.).

The data indicate that with both NE and DA the specific activities are ten to twenty times greater in the membrane enzyme fraction than in the soluble component. The actual percentage of the COMT activity found in the microsomal fraction varies considerably between the different preparations of the brain homogenates, as the data in Table 2 demonstrate. The total activity in the microsomal fraction varies anywhere from 1 to 3.5 times greater than the activity in the soluble fraction.

The results reported in Table 1 also indicate that differences in the rate of metabolism of DA and NE exist when comparing the membrane-bound and soluble fractions of human brain COMT. At 2 µM, DA was O-methylated at approximately four times the rate of NE, whereas the difference in this rate with the soluble enzyme fraction was less than two. These data

suggest that the affinities for DA and NE may be different in the microsomal and soluble tissue fractions. Accordingly, studies were performed to determine the K_m values

Table 2. Percentage of microsomal and soluble COMT in human brain*

Expt.	Microsomal/Soluble Ratio
1	3.5
2	1.1
3	1.3
4	2.8

* Concentration of dopamine was 2 μM. Total activity in the microsomal fraction varied from 0.3 to 1.3 nmoles product formed/30 min.

for DA with soluble and membrane-bound COMT. The Lineweaver-Burk plot shown in Fig. 1A illustrates that only a single line results from O-methylation of DA when the microsomal fraction is used. The K_m value for membrane-bound COMT resulting from the average of three experiments was approximately 3 μM. In contrast, the data plotted in Fig. 1B indicate that the soluble fraction consistently produced a biphasic graph yielding two K_m values of approximately 5 and 83 μM.

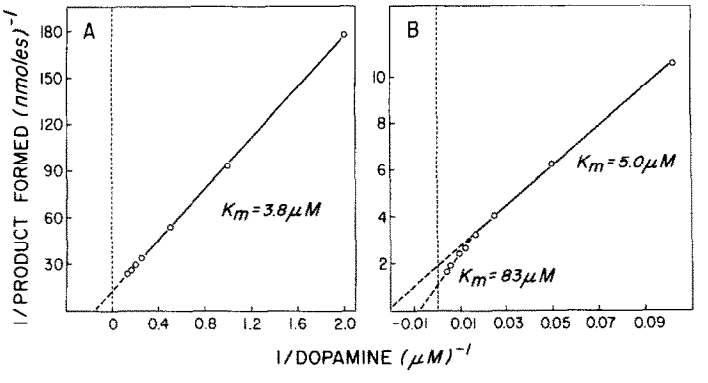

Fig. 1. Lineweaver-Burk plots for O-methylation of dopamine by membrane-bound (A) and soluble COMT (B) from human brain.

The results of this study demonstrate that a major portion of human brain COMT activity is localized in the microsomal fraction. Thus, human brain is similar to human lymphocytes in that the membrane-bound species of COMT makes up a major portion of the total transferase activity [3]. The soluble fraction that was examined in this study contained two distinct forms of COMT. One of the two forms is likely a small soluble microsomal fragment containing the membrane-bound enzyme since this component had a K_m value very similar to that of the microsomal COMT. The other molecular species resembles the soluble form of COMT as previously characterized by White and Wu [8]. Since the properties of detergent solubilized rat brain membrane-bound COMT closely resemble those of the cytosolic or soluble enzyme, the results obtained here with human brain demonstrate

for the first time that a soluble species of this enzyme, though not detergent solubilized, is also capable of resembling the membrane-bound enzyme.

Several studies [4,6,7] have reported that the affinities of catechol substrates for rat membrane-bound COMT are considerably greater than those for the cytosolic enzyme. The results with human brain COMT are consistent with this finding in that the affinity of DA for the microsomal enzyme is approximately 25 times greater than for the membrane-bound COMT. Since the activity of the membrane-bound enzyme represents a major portion of the total COMT activity, it is unlikely that this activity in the microsomal fraction is caused by an artifact of the soluble enzyme being bound nonspecifically to membranous material as suggested previously for the membrane-bound pig brain enzyme [11]. In support of this are preliminary studies which demonstrate that the specific activity of human brain microsomal COMT remains constant even after repeated washings. Studies are presently underway in our laboratory to further characterize the membrane-bound form of human brain COMT.

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REFERENCES

1. J. R. Aprille and D. F. Malamud, *Biochem. biophys. Res. Commun.* 64, 1293 (1975).
2. R. T. Borchardt and C. F. Cheng, *Biochim. biophys. Acta* 522, 49 (1978).
3. S. Sladek and R. Weinshilboum, *Pharmacologist* 22, 237 (1980).
4. J. H. Tong and A. D'Iorio, *Can. J. Biochem.* 55, 1108 (1977).
5. C. R. Creveling, R. T. Borchardt and C. Isersky, in *Frontiers of Catecholamine Research* (Eds. E. Usdin and S. Snyder), pp. 107-12. Pergamon Press, New York (1973).
6. M. Assicot and C. Bohuon, *Biochimie* 53, 871 (1971).
7. M. Roffman, T. G. Reigle, P. J. Orsulak and J. J. Schildkraut, *Biochem. Pharmac.* 25, 208 (1976).
8. H. L. White and J. C. Wu, *Biochem. J.* 145, 135 (1975).
9. S. Wrenn, C. Homey and E. Haber, *J. biol. Chem.* 254, 5708 (1979).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* 193, 265 (1951).
11. R. Goldberg and K. F. Tipton, *Biochem. Pharmac.* 27, 2623 (1978).