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PRELIMINARY COMMUNICATIONS

THE SITE OF O-METHYLATION BY MEMBRANE-BOUND CATECHOL-O-METHYLTRANSFERASE

Erkki Nissinen
Orion Pharmaceutical Co., Research Center
P.O. Box 8, SF-2101 Espoo 10, Finland

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It is now well documented that catechol-O-methyltransferase (COMT; EC 2.1.1.6) is present both as soluble and as membrane-bound forms in a variety of tissues such as brain (1, 2), heart (2), liver (2, 3) and red blood cells (4). The major reported distinction between these two forms is that the membrane-bound COMT has at least one order of magnitude greater affinity for the catechol substrates than the soluble COMT. Other properties of these two enzyme forms closely resemble each other (2, 3, 5).

The site of O-methylation *in vivo* is almost exclusively the *meta* hydroxyl of the catechol ring (6). However, when assayed *in vitro* COMT can methylate either hydroxyl of catechols producing significantly lower *meta* to *para* ratios than what is believed to exist *in vivo* (7). The *para* methoxy metabolites are absent or detectable at very low levels in mammalian tissues (8). Studies on the factors affecting *meta* and *para* O-methylation of catechols have suggested that the *meta*/*para* ratio depends on pH, concentration of the divalent cation and the nature of the substituent on the catechol ring (9).

The *meta* to *para* ratios of soluble and membrane-bound COMT have been reported to be similiar (9, 10) and no futher attention has been paid to this issue. However, as described in this communication we have found that membrane-bound COMT methylates catechol substrates *in vitro* at *meta* to *para* ratio corresponding *in vivo* conditions and that *meta*/*para* ratios of different tissues differ considerably from each other.

METHODS

Brains and other tissues from rats killed by carbon dioxide were homogenized 1:10 (w/v) in 10 mM potassium phosphate, 0.5 mM dithiothreitol pH 7.4 using glass homogenizer. The homogenates were centrifuged at 700 g for 10 min, and the supernatants were centrifuged at 10 000 g for 30 min. The resulting supernatants were centrifuged at 100 000 g for 60 min. The pellets

were washed twice in the homogenization buffer and recentrifuged at 100 000 g for 60 min. The membrane-bound (MB) COMT activity was assayed in the washed microsomal pellets and the soluble (S) COMT in the first 100 000 g supernatant solutions. All operations were carried out at +4°C.

COMT activity and determination of meta/para ratios was assayed by a modification of our previously described high-performance liquid chromatography with electrochemical detection (HPLC-EC) method (11). The reaction mixture contained 50 mM sodium phosphate buffer pH 7.4, 2.5 mM magnesium chloride, 0.1 mM S-adenosyl-L-methionine (SAM), 0.5 mM Pargyline, 100 ul microsomal or soluble enzyme and various concentrations of dopamine (DA) or dihydroxybenzoic acid (DBA) in a final volume of 250 ul. After 30 min incubation at 37°C reactions were terminated by the addition of 25 ul cold 4 M perchloric acid. Protein precipitate was removed by centrifugation and a 20 ul aliquot was injected into the liquid chromatograph connected to electrochemical detector set at + 0.9 V potential. The meta and para O-methylated reaction products were eluted from the 5 um ODS column with 20 % methanol containing 0.1 M sodium phosphate, 20 mM citric acid, 0.15 mM sodium EDTA and 2 mM sodium octonesulfonate pH 3.2. Protein determinations were carried out using Bio-Rad protein assay kit.

RESULTS AND DISCUSSION

The meta/para ratios of the methylated products from COMT assays using membrane-bound and soluble enzyme preparations from various rat tissues are presented in Table 1.

Table 1. Methylation ratio of meta and para hydroxyls by membrane bound and soluble COMT

		Meta: para ratios (± S.E.M., n = 6)	
Tissue		Dopamine	Dihydroxybenzoic acid
Brain	MB	61.0 ± 4.0	23.7 ± 0.9
	S	4.7 ± 0.2	5.1 ± 0.1
Liver	MB	21.5 ± 4.7	11.7 ± 0.9
	S	3.7 ± 0.1	5.7 ± 0.1
Kidney	MB	25.7 ± 2.2	7.8 ± 0.5
	S	3.7 ± 0.1	5.6 ± 0.1
Lung	MB	39.0 ± 2.0	12.8 ± 1.1
	S	4.4 ± 0.2	5.7 ± 0.1
Heart	MB	n.d.	24.6 ± 1.1
	S	4.2 ± 0.4	5.5 ± 0.2

n.d. = not detectable

The data show that with both DA and DBA as substrates MB-COMT produces significantly higher meta/para ratios than S-COMT in all tissues. When DA (50 uM) is the substrate MB-COMT yields 6 - 12 times higher meta/para ratios than the soluble form. The highest meta methylation was found in the brain and lowest in the liver. Membrane-bound enzyme from the heart had so low activity towards 50 uM dopamine that no para methylated product could be detected. When 50 uM DBA was the substrate the highest meta/para ratio was found in the heart and the lowest in the kidney.

The meta/para ratios produced by soluble COMT did not differ significantly from tissue to tissue. The values were higher with DBA than with DA but our results are in close agreement with several previous reports (9, 12, 13). The amount of the cofactor SAM did not have distinct effect on the meta/para ratios produced by S- or MB-COMT. However, a change in the substrate concentration yielded significant differences in meta/para ratios of MB-COMT as shown in Fig. 1.

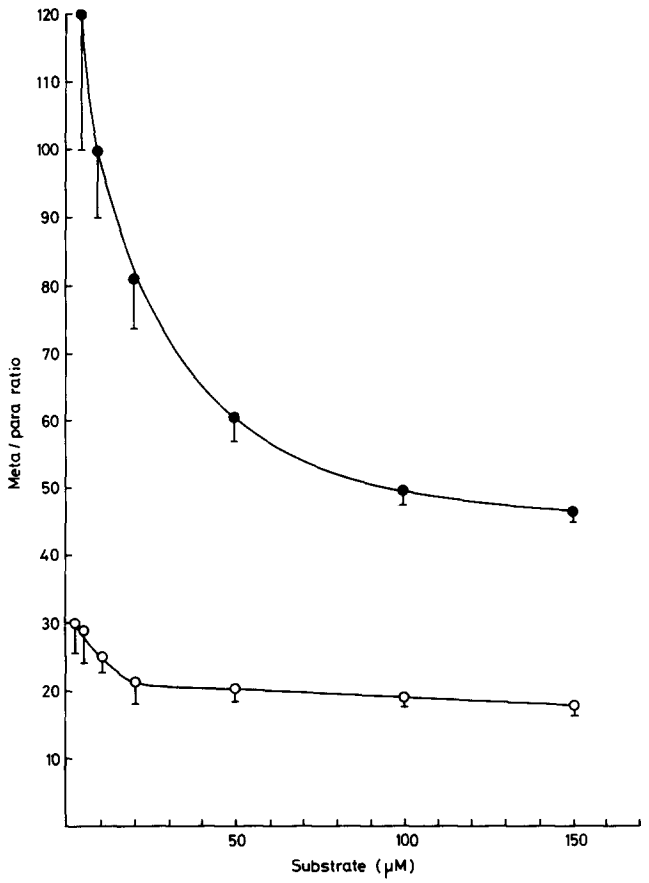

Fig 1. Meta/para ratios (± S.E.M., n = 3) for O-methylation of dopamine (●) and dihydroxybenzoic acid (o) by rat brain membrane-bound catechol-O-methyltransferase.

Low substrate concentrations yield meta/para ratios similiar to those believed to occur in vivo. The meta to para ratios produced by rat brain soluble COMT did not change significantly within the used concentration range of 2.5 - 400 μM dopamine from the average of 4.5. These findings are a futher indication that in the brain soluble and membrane-bound COMT are likely to have different functions as suggested by others (15).

From Lineweaver-Burk plots the K_m value for brain MB-COMT resulting from three experiments was 5.7 μM and for brain S-COMT 926 μM when using dopamine as the substrate within range of 2.5 - 20 μM and 50 - 400 μM respectively. These K_m values are similiar to those reported previously (14)

It has been reported that soluble COMT is present in two forms designated A and B (15), COMT-B from rat liver produces meta/para methylation ratios with DBA, which are similiar to our membrane-bound values, but with DA our values are four times higher. We feel that the soluble COMT-B is microsomal contamination in the soluble preparation, although membrane-bound and soluble COMT have same molecular weights (2) similiar to COMT-A (15) differing from the molecular weight reported for COMT-B (15).

The results of this study demonstrate that the meta/para O-methylation ratios produced by membrane-bound COMT differ significantly from meta/para ratios produced by soluble enzyme. The meta/para ratios obtained with various tissues also show differences which may be due to multiple molecular forms of membrane-bound COMT. Based on these preliminary findings studies in our laboratory are underway to futher characterize the membrane-bound COMT from various tissues.

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