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RESEARCH ARTICLE



## Delayed O-methylation of L-DOPA in MB-COMT-deficient mice after oral administration of L-DOPA and carbidopa

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### Abstract

1. Catechol-O-methyltransferase (COMT) is involved in the O-methylation of L-DOPA, dopamine, and other catechols. The enzyme is expressed in two isoforms: soluble (S-COMT), which resides in the cytoplasm, and membrane-bound (MB-COMT), which is anchored to intracellular membranes.
2. To obtain specific information on the functions of COMT isoforms, we studied how a complete MB-COMT deficiency affects the total COMT activity in the body, peripheral L-DOPA levels, and metabolism after L-DOPA (10 mg kg<sup>-1</sup>) plus carbidopa (30 mg kg<sup>-1</sup>) administration by gastric tube in wild-type (WT) and MB-COMT-deficient mice. L-DOPA and 3-O-methyl-L-DOPA (3-OMD) levels were assayed in plasma, duodenum, and liver.
3. We showed that the selective lack of MB-COMT did not alter the total COMT activity, COMT enzyme kinetics, L-DOPA levels, or the total O-methylation of L-DOPA but delayed production of 3-OMD in plasma and peripheral tissues.

### Keywords

COMT isoforms, knock-in mutation, L-DOPA, MB-COMT, metabolism, mouse pharmacokinetics, S-COMT, site-directed mutation

### History

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### Introduction

Catechol-O-methyltransferase (COMT) catalyzes the conversion of catecholamines and other catechols, e.g. L-DOPA and catechol estrogens, into their O-methylated metabolites using S-adenosyl-L-methionine as the methyl donor (Guldberg & Marsden, 1975; Männistö & Kaakkola, 1999). The COMT gene (*COMT*) codes for two isoforms of the COMT enzyme protein, soluble (S-COMT), and membrane-bound (MB-COMT) (Lundström et al., 1991; Salminen et al., 1990). The latter protein is slightly larger because it incorporates 50 additional hydrophobic amino acids that form the membrane anchor (Lundström et al., 1991; Bertocci et al., 1991). *COMT* contains six exons, the first two of which are non-coding. In exon 3, there are two AUG start codons for two promoters that control the expression of the two COMT transcripts (Tenhunen et al., 1994). The distal P2 promoter regulates the synthesis of a 1.5-kb transcript in humans. Based on the leaky scanning mechanism of translation initiation, this longer transcript can code for both S-COMT and MB-COMT proteins (Tenhunen & Ulmanen, 1993; Tenhunen et al., 1993, 1994). The P1 promoter almost completely overlaps exon 3 and falls between the S-COMT and MB-COMT ATG start codons, partially overlapping the MB-COMT coding

sequence. Therefore, the shorter mRNA transcript (1.3 kb in humans) regulated by P1 only codes for the S-COMT protein.

S-COMT and MB-COMT share the same enzymatic mechanism, but their kinetic parameters for catecholamine neurotransmitters are different. S-COMT shows high  $K_m$  values but also high  $V_{max}$  values, suggesting that S-COMT is the predominant isoenzyme under conditions where substrate concentrations are high, such as during detoxification of exogenous catechol compounds in the liver and gut wall (Guldberg & Marsden, 1975; Männistö & Kaakkola, 1999; Roth, 1992). MB-COMT, on the contrary, has low  $V_{max}$  values as well as a higher affinity to catechol substrates than S-COMT, implying a role in catecholamine neurotransmitter metabolism under low, evidently physiological concentrations (Bai et al., 2007; Lotta et al., 1995; Masuda et al., 2006; Roth, 1992). Nevertheless, differences in these kinetic parameters are not universal for all substrates. In the case of many catechol compounds, such as L-DOPA, 3,4-dihydroxybenzoid acid (DHBAC) and catecholestrogens, the affinity and rate of enzymatic reaction are similar for both isoenzymes (Lotta et al., 1995; Reid et al., 1986).

COMT is expressed widely in tissues throughout the body. The highest levels of expression are found in the liver and kidneys (Guldberg & Marsden, 1975; Männistö & Kaakkola, 1999; Myöhänen & Männistö, 2010). Distribution of COMT activity does not fully parallel COMT protein levels, suggesting that the activity is regulated at the tissue level. In addition, the S-COMT/MB-COMT ratio varies between

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tissues, which likely affects the relation between combined COMT expression levels and enzyme activity (Myöhänen & Männistö, 2010). Generally, the importance of MB-COMT-mediated O-methylation is higher in the brain than in the periphery, and S-COMT expression and activity levels are higher in peripheral tissues than in the brain (Männistö & Kaakkola, 1999; Myöhänen et al., 2010). Since estrogens down-regulate the expression of both COMT isoforms, COMT activity is generally lower in females than males although not in all tissues (Schendzielorz et al., 2011; Xie et al., 1999). Significance of the COMT gene to sex differences in brain function and predisposition to psychiatric disorders has remained unsolved (Tunbridge & Harrison, 2011).

The dopamine precursor L-DOPA, combined with an aromatic acid decarboxylase (AADC, DDC, dopa decarboxylase) inhibitor like carbidopa, is the most important component of treatment for Parkinson's disease (PD). COMT inhibitors, such as entacapone and tolcapone, are used in PD to improve further the bioavailability of L-DOPA and prolong its action (Männistö & Kaakkola, 1999).

We have developed a novel genetically engineered mouse strain that selectively lacks MB-COMT (Tammimäki et al., 2016). These mice had a distinct phenotype with increased aggressiveness, reduced prepulse inhibition, and prolonged immobility time in tail suspension test in male mice and sensitization to pain and worsened short-term memory in both sexes. Earlier, we found in S-COMT-deficient mice that the total COMT activity was decreased by about 30% in all tissues studied (Käenmäki et al., 2009). However, after L-DOPA/carbidopa administration, the levels of L-DOPA either in plasma or peripheral tissues were only slightly increased, although the levels of the O-methylated metabolite of L-DOPA, 3-OMD, were significantly decreased. Based on that, we hypothesized that overall COMT activity would be decreased in MB-COMT-deficient mice and that the peripheral metabolism of L-DOPA would be hampered as well.

In the present study, L-DOPA kinetics after oral administration of L-DOPA with carbidopa was explored. We measured L-DOPA and 3-OMD levels in plasma and peripheral tissues of the WT and MB-COMT-deficient mice. These studies provide new information on the role of COMT isoforms in L-DOPA metabolism in mice.

## Materials and methods

### Materials

L-DOPA (levodopa), 3-OMD, and 3,4-dihydroxybenzoid acid (DHBAC) were purchased from Sigma Chemical Co. (St. Louis, MO), and carbidopa was obtained from Orion Pharma (Espoo, Finland). Carboxymethyl cellulose and EDTA were purchased from Fluka Chemie (Steinheim, The Netherlands).

### Animals

MB-COMT knock-in mutant mice were generated as described elsewhere (Tammimäki et al., 2016). Briefly, a knock-in approach was used that allowed site-directed mutagenesis on the whole animal level. Two-point mutations were introduced in exon 2 of the *Comt* gene (ATGCTG

→GAGCTC) altering just one amino acid, methionine, to glutamic acid. Consequently, the function of the P2 promoter is disabled and only the P1-regulated S-COMT transcription remains.

MB-COMT-deficient male and female mice as well as their WT littermates were used for the animal experiments. The mice were bred in a SPF barrier unit at the Laboratory Animal Centre of University of Helsinki, Finland. They were weaned, sexed, and earmarked at the age of 3 weeks. After weaning, they were group housed in clear polycarbonate individually ventilated filter-top cages with aspen chip bedding and nesting material under 12:12 light cycle at an ambient temperature of 22 °C with drinking water and mouse chow available ad libitum. The mice were kept in the home cages with their same-sex littermates until sacrificed. Mice (in total 146 mice) of the sixth to seventh generations of heterozygous mating pairs were used for the experiments; C57BL/6JHsd mice were regularly included in the breeding to maintain the strain on that particular background. Experimentally naïve animals entered the experiments when they were two to three months old and weighed 20–30 g. The phase of the estrus cycle was not determined. In all experiments, MB-COMT-deficient male and female groups were compared with respective WT control groups. In the end of experiments, the animals were sacrificed by cervical dislocation.

Animal experiments were conducted according the 3R principles of the EU directive 2010/63/EU governing the care and use of experimental animals, and following local laws and regulations [Finnish Act on the Protection of Animals Used for Scientific or Educational Purposes (497/2013), Government Decree on the Protection of Animals Used for Scientific or Educational Purposes (564/2013)]. The protocols were authorized by the national Animal Experiment Board of Finland.

### Genotyping of MB-COMT-deficient knock-in mice

Genotype was determined from tissue samples obtained during earmarking of the mice. Genomic DNA was extracted from the ear biopsies as described by Laird et al. (1991). PCR mix consisted *Comt3HAUF2* (oligo No: 40612F3A03 1/4, sequence: 5'-GAAGTGGGTATGGCAGCGCTTATA-3') and *Comt5HADR2* (oligo no. 40612F3B03 2/4 sequence: 5'-AACACACATTCCTCTC-ATGCTCCT-3') primers from Oligomer (Helsinki, Finland) and Fail safe system premix B. The amplified fragments were visualized by SyBr Green (Qiagen, Venlo, The Netherlands) staining under ultraviolet light after electrophoresis in a 1.7% agarose gel.

### COMT activity assay

Livers for total COMT activity assay were collected from MB-COMT-deficient and WT mice of both sexes. The tissues were frozen on dry ice and stored at –80 °C before analysis. Detailed method of the COMT activity assay is given elsewhere (Käenmäki et al., 2009). Activity data are given as picomoles of vanillic acid (and isovanillic acid for meta/para calculation) formed per minute per milligram of tissue. Liver COMT activities were measured with graded concentrations of DHBAC (1, 10, and 100 μM) and enzyme kinetics

( $K_m$  and  $V_{max}$ ) were calculated with GraphPad Prism 5.0. (San Diego, CA).

### Western blotting

For Western immunoblotting (WB), the tissue samples (liver and duodenum) were collected from at least two male and two female mice and rinsed in physiological saline solution. Immediately after dissection, the tissues were placed in ice-cold centrifuge tubes on dry ice to minimize decomposition. All the tissue samples were frozen and stored in  $-80^{\circ}\text{C}$  until analyzed. Tissues were lysed in 10 volumes of RIPA lysis buffer (20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 1% NP-40, 2 mM EDTA) containing protease and phosphatase inhibitors, homogenized manually, incubated for 20 min on ice and centrifuged ( $18\,000 \times g$  for 15 min at  $4^{\circ}\text{C}$ ). The supernatants were resolved by electrophoresis on a 4–20% precast gel (Mini Protean TGX Gel, Biorad, Hercules, CA). Proteins were transferred onto Trans-Blot Turbo Nitrocellulose filter and transferred using the Trans-Blot Turbo Transfer system (Biorad, Hercules, CA). The membranes were blocked with 0.1% (w/w) Tween-20/TBS containing 5% (w/w) non-fat-dried milk at room temperature for 1 h. After blocking, the membranes were incubated overnight with mouse anti-COMT antibody (1:8000, BD Biosciences, Franklin Lakes, NJ) and mouse anti  $\beta$ -actin antibody (1:10 000, clone AC-74, purified immunoglobulins, 107K4791, Sigma-Aldrich Inc., MO) followed by incubation with goat anti-mouse secondary antibody, HRP conjugated (1:2000, Thermo Fisher Scientific, Waltham, MA) for 1 h at room temperature. The membranes were incubated with ECL detection reagent (Thermo Fisher Pierce, Rockford, IL) for 5 min to visualize proteins, and then visualized using C-Digit blot scanner (Li-COR, Lincoln, NE). Blots were analyzed using ImageJ freeware (NIH, Bethesda, MD).

### Pharmacokinetics of L-DOPA

The mice were given L-DOPA ( $10\text{ mg kg}^{-1}$ ) and carbidopa, ( $30\text{ mg kg}^{-1}$ ) suspended in 0.25% carboxymethyl cellulose gel (volume  $5\text{ ml kg}^{-1}$ ) with a silicon-tipped stainless steel gastric tube. The drug doses were chosen to match the ones used in our previous L-DOPA studies (Huotari et al., 2002; Käenmäki et al., 2009). From each mouse, one baseline blood sample and two blood samples after L-DOPA administration were collected in Microvette capillary blood collection tubes containing potassium EDTA (Sarstedt, Nümbrecht, Germany) at time points 30, 60, and 120 min. The first two samples ( $100\ \mu\text{l}$ ) were taken from the saphenous vein (<https://www.nc3rs.org.uk/mouse-saphenous-vein-non-surgical>) and the third, terminal sample (approximately  $300\ \mu\text{l}$  of the whole trunk blood) by decapitation of the cervically dislocated animals. The blood samples were centrifuged at  $4900 \times g$  at  $4^{\circ}\text{C}$  for 10 min. We aimed to collect six independent samples of each genotype at every time point was measured. After the terminal blood sample (at 60 or 120 min) was collected by decapitation, pieces of the liver and duodenum were dissected and frozen on dry ice. Control tissues (shown as time point 0 in results) were collected from drug-naïve mice. Plasma and tissues were stored at  $-80^{\circ}\text{C}$  until assays.

Plasma (Käenmäki et al., 2009) and tissue (Airavaara et al., 2006; Huotari et al., 2002) samples were prepared as previously described and L-DOPA and 3-OMD were quantified from the filtrates using HPLC with electrochemical detection as described in (Käenmäki et al., 2009). The values are presented as nanograms per mg of wet tissue weight or nanograms per ml of plasma. The number of animals per group in these analyses was 4–7. In our experience, this number is large enough to show potential significant differences between groups. There were seven animals in male WT group at 0 min, six animals in male MB-COMT-deficient mice at 0 min, female WT mice at 120 min, female MB-COMT-deficient mice at 60 min and female MB-COMT-deficient mice at 120 min groups and four animals in female WT 0 min group. Rest of the groups had five animals. Four tissue samples were excluded due to failed sample preparation or analysis. Thus, in these groups, there are fewer replications than in others (male WT mice at 60 min, female MB-COMT-deficient mice at 30 and 60 min).

### Statistics

SPSS 22 statistic software (SPSS Inc., Chicago, IL) was used for all calculations. The results are shown as mean  $\pm$  standard error of mean (SEM). AUC values were calculated employing the trapezoidal rule (calculated of time points between 0 and 120 min).

Two-way ANOVA for repeated measures with sex and genotype as independent variables was used to test the L-DOPA kinetic data and COMT enzyme activity. If the initial two-way ANOVA showed a significant sex effect, the data were further analyzed with one-way ANOVA for repeated measures. Differences in L-DOPA/3-OMD ratios at each time point were analyzed by two-way ANOVA and meta/para ratios with one-way ANOVA followed by Newman-Keuls test.

### Results

#### COMT proteins in the liver and duodenum

Representative Western blots from duodenum and liver of male and female WT and MB-COMT-deficient mice (Figure 1) show that the latter express S-COMT protein, while no MB-COMT protein is present. The amount of MB-COMT is about one-tenth of S-COMT. We did not see any

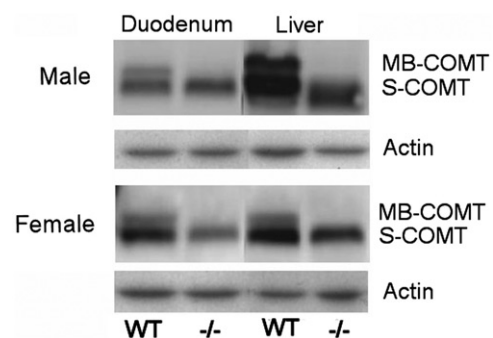


Figure 1. Western blot analysis of the liver and duodenum showing a complete lack of MB-COMT protein in the mutated mice. WT: wild-type, -/-: MB-COMT-deficient mice.

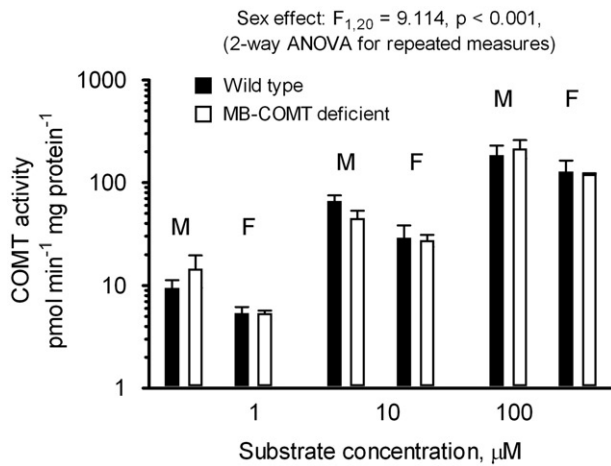


Figure 2. Total COMT activity in the liver (mean  $\pm$  SEM,  $n = 4\text{--}5$ /group) measured at three substrate (DHBAC) concentrations (1, 10, and 100  $\mu\text{M}$ ). There was a significant sex effect females having generally lower COMT activity than males. However, the lack of MB-COMT did not alter the total COMT activity.

upregulation of S-COMT as a reaction to the lacking MB-COMT.

### Total COMT activity in the liver

As expected, increased total COMT activity in the liver (Figure 2) was observed with increasing concentrations of the substrate without any significant difference between the values in the WT and MB-COMT-deficient mice. There were no significant differences in the kinetic values between genotypes and sexes calculated from the present substrate concentrations (1, 10, and 100  $\mu\text{M}$ ) and those measured earlier at 3, 30, and 300  $\mu\text{M}$  (Tammimäki et al., 2016):  $V_{\text{max}}$ : in males,  $481 \pm 65$  versus  $385 \pm 57 \text{ min}^{-1}$  in WT and MB-COMT-deficient mice, and in females,  $381 \pm 113$  versus  $536 \pm 110 \text{ min}^{-1}$ , respectively;  $K_m$ : in males,  $115 \pm 56$  versus  $89 \pm 27 \mu\text{M}$ , and in females,  $180 \pm 79$  versus  $277 \pm 75 \mu\text{M}$ , in WT and MB-COMT-deficient mice, respectively.

However, there was a sex difference in liver COMT activity across different substrate concentrations [two-way ANOVA for repeated measures:  $F_{2,40} = 8.645$ ,  $p < 0.01$ ]. Meta/para ratios were always less than 15, typical to S-COMT activity, at all substrate levels. In all material, meta/para ratios of the males were  $12.0 \pm 1.4$  (WT) and  $11.6 \pm 1.7$  (MB-COMT deficient) and in females,  $6.7 \pm 0.4$  and  $6.5 \pm 0.4$ , respectively. Female values were significantly lower than male values [one-way ANOVA:  $F_{3,50} = 6.81$ ,  $p < 0.01$ ].

### Pharmacokinetics of L-DOPA

#### L-DOPA and 3-OMD in plasma

Figure 3 shows the concentrations of L-DOPA and its 3-OMD in the plasma of the MB-COMT-deficient mice and their WT littermates of both sexes. Neither the sex nor the genotype affected concentrations of L-DOPA in plasma. Although there was neither a genotype- nor sex-dependent effect on 3-OMD levels, the interaction between the two was significant [two-way ANOVA for repeated measures, sex  $\times$  genotype interaction:  $F_{1,17} = 5.338$ ,  $p < 0.05$ ]. This means that genotype

had a different effect on 3-OMD levels in males than in females. In practice, male mice showed no genotype effect whereas in females the 3-OMD levels tended to be lower in MB-COMT deficient than in WT mice. Furthermore, the kinetics of the 3-OMD was altered in the MB-COMT-deficient mice, as the levels of 3-OMD did rise more slowly in the mutated mice as compared with the WT animals [two-way ANOVA for repeated measures, time  $\times$  genotype interaction  $F_{4,68} = 6.965$ ,  $p < 0.001$ ].

L-DOPA/3-OMD ratios at 30 min confirmed that O-methylation of L-DOPA was significantly delayed in both male (WT,  $4.3 \pm 1.6$  versus MB-COMT deficient,  $11.8 \pm 1.1$ ) and female ( $5.3 \pm 0.7$  versus  $14.8 \pm 1.1$ ) MB-COMT-deficient mice [two-way ANOVA: genotype effect  $F_{1,16} = 19.42$ ,  $p < 0.01$ ]. However, there was a significant overshooting of O-methylation at 120 min in MB-COMT-deficient mice (males:  $3.1 \pm 0.5$  versus  $0.5 \pm 0.2$ ; females:  $4.0 \pm 1.4$  versus  $0.5 \pm 0.08$ , for WT and MB-COMT deficient, respectively) [two-way ANOVA: genotype effect  $F_{1,18} = 6.65$ ,  $p < 0.05$ ].

#### L-DOPA and 3-OMD in tissues

Figure 4 shows that neither the gender nor the genotype significantly affected the L-DOPA levels in hepatic and duodenal tissues. In addition, neither sex nor genotype affected hepatic and duodenal concentrations of 3-OMD. However, in both tissues, genotype affected the 3-OMD levels in male mice measured over time [two-way ANOVA for repeated measures, liver: time  $\times$  genotype interaction  $F_{2,30} = 6.990$ ,  $p < 0.01$ ; duodenum: time  $\times$  genotype interaction  $F_{2,30} = 5.617$ ,  $p < 0.01$ ]. Like 3-OMD levels in plasma, also 3-OMD tissue levels appeared to increase more slowly in MB-COMT-deficient mice as compared with their WT littermates (excluding duodenal tissue in females).

### Discussion

In this study, we explored the effect of complete MB-COMT deficiency on hepatic COMT activity and L-DOPA kinetics and metabolism in mouse. We showed that the lack of MB-COMT affected neither the total COMT activity in the liver nor L-DOPA levels in but shifted the 3-OMD time-concentration curve to the right in plasma and peripheral tissues.

In our previous studies in S-COMT-deficient mice, we found a non-significant 20–30% increase of L-DOPA levels in the plasma, liver and duodenum (Käenmäki et al., 2009), while in the COMT knock-out mice, L-DOPA levels were approximately doubled as compared with the WT mice (Käenmäki et al., 2009). In the present study, we did not observe a significant overall effect of MB-COMT genotype on 3-OMD levels in the plasma and peripheral tissues (Figures 3 and 4). Instead, in the MB-COMT-deficient mice, we observed a significant delay in plasma and peripheral tissue 3-OMD levels as compared with WT animals. This was reflected in the significantly reduced L-DOPA/3-OMD ratios at 30 min in the MB-COMT-deficient mice of both sexes. At 120 min, there was an overshoot of the ratio balancing the total L-DOPA O-methylation to the level of the WT mice. In the previous study, we showed 30–40% lower 3-OMD levels in plasma, liver, and duodenum in S-COMT-deficient animals than in their WT littermates

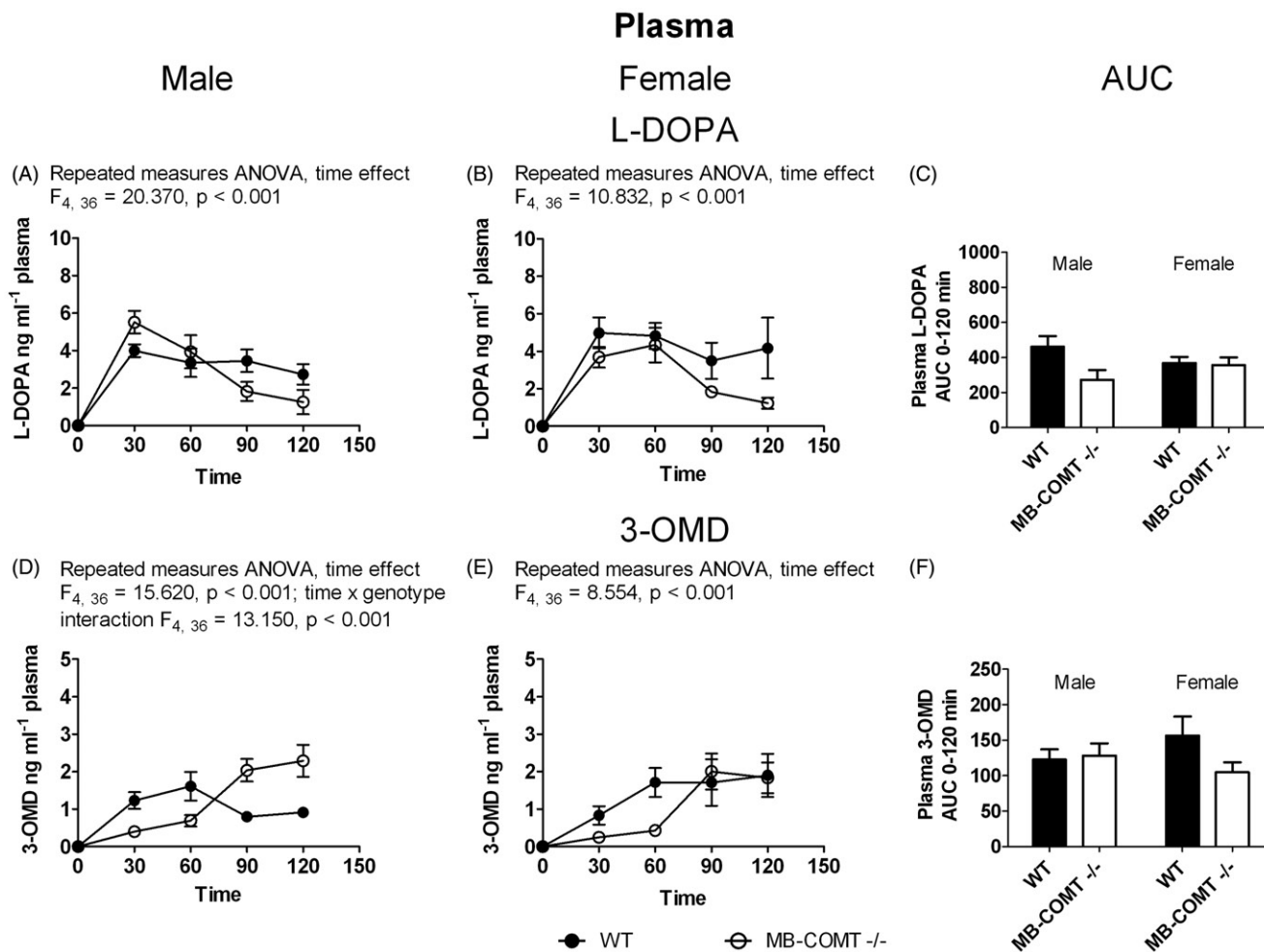


Figure 3. L-DOPA and 3-OMD in plasma. Concentrations of L-DOPA and 3-OMD in the plasma of male and female MB-COMT-deficient animals and their wild-type littermates as well as  $AUC_{0-120\text{ min}}$  values calculated based on time-concentration curves. The mice were treated with L-DOPA (10 mg/kg) and the dopa decarboxylase inhibitor carbidopa (30 mg/kg) by gastric tube and blood samples were taken at 30, 60, 90, and 120 min after drug administration. Control blood was drawn before drug administration. From each mouse, three blood samples were taken. The time points of blood sampling were randomized within each genotype and sex group. WT: wild-type, MB-COMT<sup>-/-</sup>: MB-COMT-deficient mice. One-way ANOVA for repeated measures indicated a significant time effect for both sexes in L-DOPA and 3-OMD data analyses. However, genotype  $\times$  time interaction proved to be significant only for the male 3-OMD levels. One-way ANOVA  $F$ -values are presented in the figure. Data are given as mean  $\pm$  SEM,  $n = 5-7$  per time point.

(Käenmäki et al., 2009). Altogether, these results suggest that S-COMT can effectively compensate functionally for the deficit caused by the lack of the MB-COMT isoform. The delayed initial metabolism of L-DOPA was an exception, stressing an unexpected importance of MB-COMT in the peripheral metabolism of L-DOPA where S-COMT was not fully able to compensate for the lack of MB-COMT.

Enzyme kinetic properties of pure recombinant human COMT isoforms for L-DOPA, dopamine and noradrenaline have shown that compared with S-COMT, the  $V_{\text{max}}$  (capacity) of MB-COMT for L-DOPA, DHBAC (the substrate of activity assay), dopamine, and noradrenaline is about one-half, whereas the affinity ( $K_m$ ) for L-DOPA is only two-fold (for DHBAC even less) but more than 10-fold for both catecholamines. The amount of MB-COMT protein was about 10% of that of S-COMT in the duodenum. Also in our earlier study, the amount of MB-COMT in the mouse duodenum was considerably smaller than that of S-COMT (Myöhänen et al., 2010). The relatively small affinity difference between MB-COMT and S-COMT for L-DOPA probably means that

MB-COMT is participating to a minor extent in L-DOPA O-methylation in the upper gastrointestinal tract in the WT mice and, therefore, a lack of MB-COMT may contribute to the initial delay of L-DOPA O-methylation.

Unaltered total COMT activity in the mice lacking MB-COMT could be explained by significant differences of enzyme kinetic properties of the two COMT isoforms for catecholamines (see Introduction and the preceding paragraph) as we have thoroughly discussed before (Tammimäki et al., 2016).  $K_m$  value of MB-COMT for catecholamines is less than one-tenth (or even smaller in some studies, Roth, 1992) of the corresponding value of S-COMT. In addition, the meta/para ratio suggests dominance of O-methylation by S-COMT even in WT mice (less than 15) and this ratio was not altered by a complete lack of MB-COMT. Considering the dominance of behavioral effects of MB-COMT deficiency in males (Tammimäki et al., 2016), it is notable that meta/para ratios were significantly higher in males than females, suggesting an important role for MB-COMT, particularly in males. There is no doubt that S-COMT is the dominating

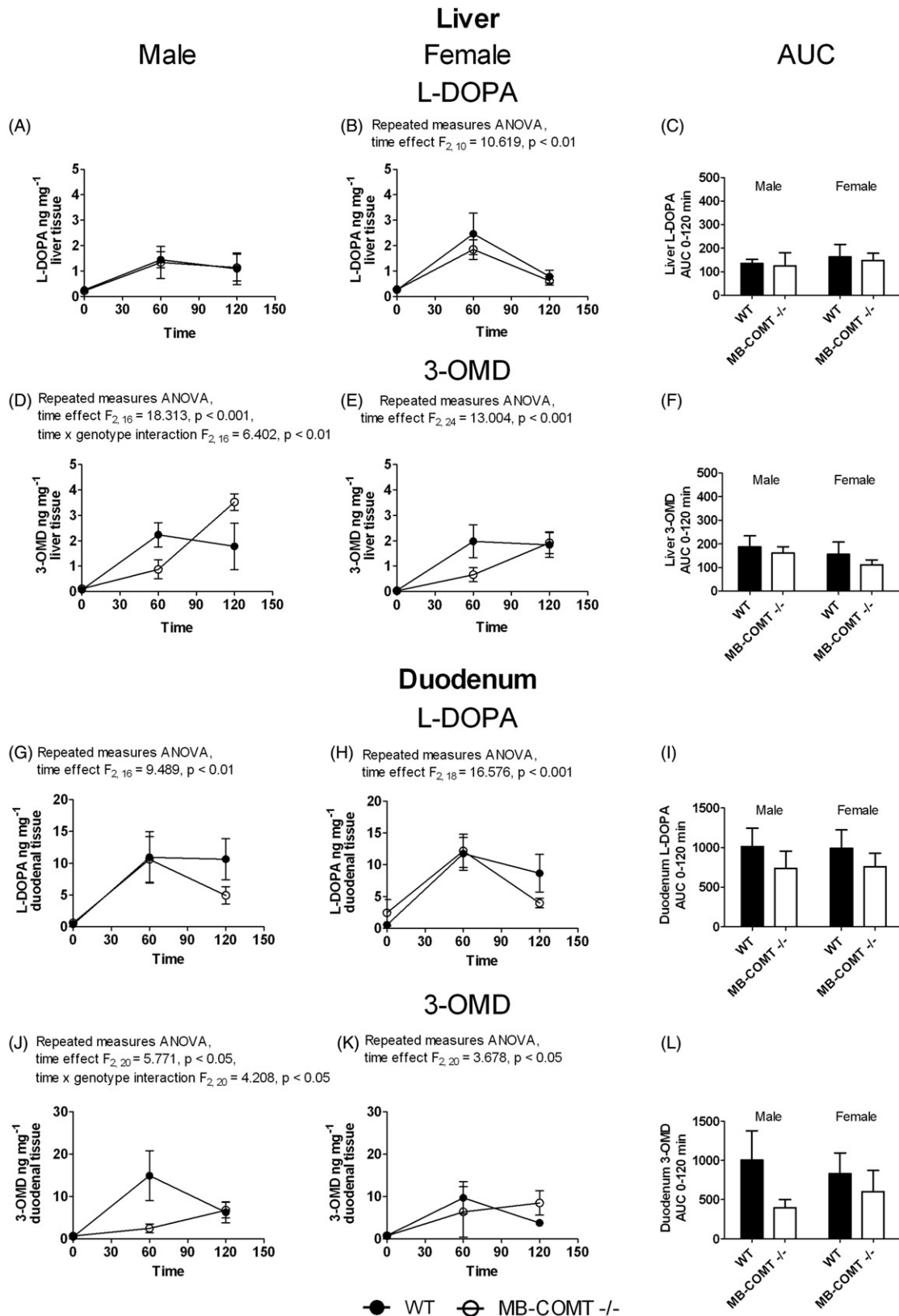


Figure 4. L-DOPA and 3-OMD in peripheral tissues. Concentrations of L-DOPA and 3-OMD in hepatic and duodenal tissue from MB-COMT-deficient mice and their wild-type (WT) littermates as well as  $AUC_{0-120\text{min}}$  values based on time–concentration curves. The mice were treated with L-DOPA (10 mg/kg) and the dopa decarboxylase inhibitor carbidopa (30 mg/kg) by gastric tube. Mice were sacrificed 60 or 120 min after drug administration. Control tissues were collected from drug-naïve mice. WT: wild-type; MB-COMT<sup>-/-</sup> = MB-COMT-deficient mice. One-way ANOVA for repeated measures indicated a significant time effect for L-DOPA in females in the liver and in both sexes in the duodenum and 3-OMD in both sexes and tissues. However, genotype  $\times$  time interaction proved to be significant only for 3-OMD in males. One-way ANOVA  $F$ -values are presented in the figure. Data are given as mean  $\pm$  SEM,  $n = 5-7$  per time point.

enzyme of overall COMT activity in the body. It is interesting to note that in the S-COMT-deficient mice (Käenmäki et al., 2009), MB-COMT was able to maintain about 70% of the total COMT activity, suggesting that under extreme conditions MB-COMT can compensate for missing S-COMT protein. We do not report brain tissue levels of L-dopa, 3-OMD, or dopamine after L-DOPA plus carbidopa administration in this article, but after a similar treatment, we earlier found profound alterations in the extracellular dopamine levels in microdialysis studies (Tammimäki et al., 2016). Notably, dopamine levels were increased in the striatum but, instead, decreased in the prefrontal cortex in the MB-COMT-deficient mice compared with the WT mice. MB-COMT certainly has a function under normal conditions as well, and the behavioral phenotype of MB-COMT-deficient mice gives hints of its functional role in some brain areas (Tammimäki et al., 2016). However, in peripheral L-DOPA metabolism, the effect of MB-COMT can be considered marginal, and our finding does not have any eminent clinical significance. Present COMT inhibitors inhibit both isoforms of COMT (Männistö & Kaakkola, 1999) and, therefore, the peripheral distribution profile remains insignificant.

In conclusion, our initial hypothesis did not turn out to be true. In the MB-COMT-deficient mice, S-COMT is generally capable of maintaining a normal level of COMT function and enzyme kinetics. L-DOPA levels and 3-OMD formation after L-DOPA plus carbidopa administration do not change significantly in the absence of MB-COMT, but the 3-OMD concentration–time curve shifts to the right in plasma and peripheral tissues, suggesting a temporarily delayed L-DOPA O-methylation.

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## Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article. This work was supported by Academy of Finland (AT grant number 1257339, PTM grant number 257898) and Sigrid Juselius Foundation (PTM).

## References

Airavaara M, Mijatović J, Vihavainen T, et al. (2006). In heterozygous GDNF knockout mice the response of striatal dopaminergic system to acute morphine is altered. *Synapse* 59:321–9.

Bai HW, Shim JY, Yu J, et al. (2007). Biochemical and molecular modeling studies of the O-methylation of various endogenous and exogenous catechol substrates catalyzed by recombinant human soluble and membrane-bound catechol-O-methyltransferases. *Chem Res Toxicol* 20:1409–25.

Bertocci B, Miggiano V, Da Prada M, et al. (1991). Human catechol-O-methyltransferase: cloning and expression of the membrane-associated form. *Proc Natl Acad Sci USA* 88:1416–20.

Gulberg HC, Marsden CA. (1975). Catechol-O-methyl transferase: pharmacological aspects and physiological role. *Pharmacol Rev* 27: 135–206.

Huotari M, Gogos JA, Karayiorgou M, et al. (2002). Brain catecholamine metabolism in catechol-O-methyltransferase (COMT)-deficient mice. *Eur J Neurosci* 15:246–56.

Käenmäki M, Tammimäki A, García-Horsman JA, et al. (2009). Importance of membrane-bound catechol-O-methyltransferase in L-DOPA metabolism: a pharmacokinetic study in two types of COMT gene modified mice. *Br J Pharmacol* 158:1884–94.

Laird PW, Zijderveld A, Linders K, et al. (1991). Simplified mammalian DNA isolation procedure. *Nucleic Acids Res* 19:4293.

Lotta T, Vidgren J, Tilgmann C, et al. (1995). Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 34:4202–10.

Lundström K, Salminen M, Jalanko A, et al. (1991). Cloning and characterization of human placental catechol-O-methyltransferase cDNA. *DNA Cell Biol* 10:181–9.

Männistö PT, Kaakkola S. (1999). Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev* 51:593–628.

Masuda M, Tsunoda M, Imai K. (2006). Low catechol-O-methyltransferase activity in the brain and blood pressure regulation. *Biol Pharm Bull* 29:202–5.

Myöhänen TT, Männistö PT. (2010). Distribution and functions of catechol-O-methyltransferase proteins: do recent findings change the picture? *Int Rev Neurobiol* 95:29–47.

Myöhänen TT, Schendzielorz N, Männistö PT. (2010). Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. *J Neurochem* 113:1632–43.

Reid JJ, Stitzel RE, Head RJ. (1986). Characterization of the O-methylation of catechol oestrogens by intact rabbit thoracic aorta and subcellular fractions thereof. *Naunyn Schmiedebergs Arch Pharmacol* 334:17–28.

Roth JA. (1992). Membrane-bound catechol-O-methyltransferase: a reevaluation of its role in the O-methylation of the catecholamine neurotransmitters. *Rev Physiol Biochem Pharmacol* 120:1–29.

Salminen M, Lundström K, Tilgmann C, et al. (1990). Molecular cloning and characterization of rat liver catechol-O-methyltransferase. *Gene* 93:241–7.

Schendzielorz N, Rysä A, Reenilä I, et al. (2011). Complex estrogenic regulation of catechol-O-methyltransferase (COMT) in rats. *J Physiol Pharmacol* 62:483–90.

Tammimäki A, Aonurm-Helm A, Zhang, et al. (2016). Generation of membrane-bound catechol-O-methyl transferase deficient mice with distinct sex dependent behavioral phenotype. *J Physiol Pharmacol* 67: 827–42.

Tenhunen J, Salminen M, Lundström K, et al. (1994). Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters. *Eur J Biochem* 223: 1049–59.

Tenhunen J, Salminen M, Jalanko A, et al. (1993). Structure of the rat catechol-O-methyltransferase gene: separate promoters are used to produce mRNAs for soluble and membrane-bound forms of the enzyme. *DNA Cell Biol* 12:253–63.

Tenhunen J, Ulmanen I. (1993). Production of rat soluble and membrane-bound catechol O-methyltransferase forms from bifunctional mRNAs. *Biochem J* 296:595–600.

Tunbridge EM, Harrison PJ. (2011). Importance of the COMT gene for sex differences in brain function and predisposition to psychiatric disorders. *Curr Top Behav Neurosci* 8:119–40.

Xie T, Ho SL, Ramsden D. (1999). Characterization and implications of estrogenic down-regulation of human catechol-O-methyltransferase gene transcription. *Mol Pharmacol* 56:31–8.