International Journal of Neuropsychopharmacology (2014), 17, 1707–1713. © CINP 2014
doi:10.1017/S1461145714000467

ARTICLE

Methionine sulfoxide reductase regulates brain catechol-*O*-methyl transferase activity

Jackob Moskovitz[1], Consuelo Walss-Bass[2], Dianne A. Cruz[2], Peter M. Thompson[2] and Marco Bortolato[1]

[1] *Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS, 66045, USA*

[2] *Department of Psychiatry, School of Medicine, University of Texas Health Science Center, San Antonio, TX, 78229, USA*

Abstract

Catechol-*O*-methyl transferase (COMT) plays a key role in the degradation of brain dopamine (DA). Specifically, low COMT activity results in higher DA levels in the prefrontal cortex (PFC), thereby reducing the vulnerability for attentional and cognitive deficits in both psychotic and healthy individuals. COMT activity is markedly reduced by a non-synonymous single-nucleotide polymorphism (SNP) that generates a valine-to-methionine substitution on the residue 108/158, by means of as-yet incompletely understood post-translational mechanisms. One post-translational modification is methionine sulfoxide, which can be reduced by the methionine sulfoxide reductase (Msr) A and B enzymes. We used recombinant COMT proteins (Val/Met^{108}) and mice (wild-type (WT) and *MsrA* knockout) to determine the effect of methionine oxidation on COMT activity and COMT interaction with Msr, through a combination of enzymatic activity and Western blot assays. Recombinant COMT activity is positively regulated by MsrA, especially under oxidative conditions, whereas brains of *MsrA* knockout mice exhibited lower COMT activity (as compared with their WT counterparts). These results suggest that COMT activity may be reduced by methionine oxidation, and point to Msr as a key molecular determinant for the modulation of COMT activity in the brain. The role of Msr in modulating cognitive functions in healthy individuals and schizophrenia patients is yet to be determined.

Received 30 September 2013; Reviewed 8 November 2013; Revised 10 March 2014; Accepted 17 March 2014; First published online 15 April 2014

Key words: Catechol-*O*-methyltransferase, methionine oxidation, oxidative stress, post-translation modification, prefrontal cortex.

Introduction

The enzyme catechol-*O*-methyltransferase (COMT) catalyzes the *O*-methylation of catecholamine neurotransmitters, such as dopamine (DA) and norepinephrine (Axelrod and Tomchick, 1958), using S-adenosylmethionine (SAM) as a methyl donor (Männistö and Kaakkola, 1999). The COMT protein occurs as two distinct isoforms with identical kinetic mechanisms, which result from differential transcriptions of the corresponding gene: a soluble form (S-COMT), found in the cell cytoplasm; and a membrane-bound form (MB-COMT), which features 50 additional residues in the *N*-terminal portion of the protein (Huh and Friedhoff, 1979; Männistö and Kaakkola, 1999).

Several lines of evidence have shown that COMT serves a primary role in DA degradation in the prefrontal cortex (PFC) (Karoum et al., 1994; Matsumoto et al., 2003a, b). Given the well-documented implication of DAergic neurotransmission in the modulation of these tasks (Miller and Cohen, 2001; Cohen et al., 2002; Nieoullon, 2002), the functional role of COMT in the PFC has garnered substantial interest. In particular, numerous investigations have focused on rs4680, one of the best-characterized single-nucleotide polymorphisms (SNPs) of the *COMT* gene, resulting in the substitution of a valine (*Val*) for a methionine (*Met*) residue at position 108 of S-COMT and 158 of MB-COMT (*Val$^{108/158}$Met*) (Lachman et al., 1996). The *Met* allele is associated with reduced COMT activity and lower DA metabolism; this functional characteristic has been shown to lead to a more efficient physiological response in the PFC across several functional domains, including cognitive flexibility, working memory, attentional control and emotional resilience (Malhotra et al., 2002; Goldberg et al., 2003; Blasi et al., 2005; Smolka et al., 2005).

The notion that low DAergic activity in the PFC may contribute to negative and cognitive symptoms of schizophrenia patients (Davis et al., 1991; Kahn and Davis, 1995), has also led several authors to investigate the potential influence of the rs4680 polymorphism of

Address for correspondence: J. Moskovitz, Department of Pharmacology and Toxicology, University of Kansas, 1251 Wescoe Hall Dr., Lawrence, KS 66045, USA.
Tel.: 785-864-3536 *Fax*: 785-864-5219
Email: moskovij@ku.edu

COMT on the severity of these deficits in psychotic disorders. Multiple studies have ascertained that the *Met^108/158^* variant is associated with a slightly lower schizophrenia risk, as well as less severity of attentional, cognitive and information-processing deficits (Egan et al., 2001; Bilder et al., 2002; Bray et al., 2003; Gallinat et al., 2003; Tunbridge et al., 2006; Ehlis et al., 2007).

Previous research has shown that the *Val$^{108/158}$Met* genotype affects protein expression, but not messenger RNA (*mRNA*) expression levels, indicating that this polymorphism may lead to post-translational changes in the protein (Matsumoto et al., 2003a, b; Chen et al., 2004). Indeed, it has been shown that the methionine-to-valine substitution reduces the thermostability of the enzyme without affecting its structural and kinetic properties (Lotta et al., 1995; Lachman et al., 1996). Although the bases of this phenomenon are not fully elucidated, the lower catalytic activity of the *Met$^{108/158}$* variant has been associated with a markedly higher susceptibility to oxidation (Cotton et al., 2004). The most oxidation-sensitive amino acids are the sulfur-containing methionine and cysteine. Indeed, Cotton and co-workers (Cotton et al., 2004) identified a key role of cysteine residues in the vulnerability of the *Met$^{108/185}$* variant to oxidation; the specific role of methionine in this process, however, remains elusive. The oxidation of methionine residues leads to the formation of methionine sulfoxide (MetO); this well-known phenomenon is enhanced by oxidative stress and reversed by the methionine sulfoxide reductase (Msr) system, consisting of two enzymes (termed A and B) that reduce either *S or R* enantiomers of MetO, respectively (Moskovitz et al., 2000, 2002; Moskovitz, 2005).

In this study, we hypothesized that the lower catalytic activity of the *Met$^{108/158}$* variant may be also contributed by the oxidation of this and other methionine residues. To test for this possibility, we assessed whether COMT activity may be affected by Msr, using a number of complementary *in vitro* and *ex vivo* approaches.

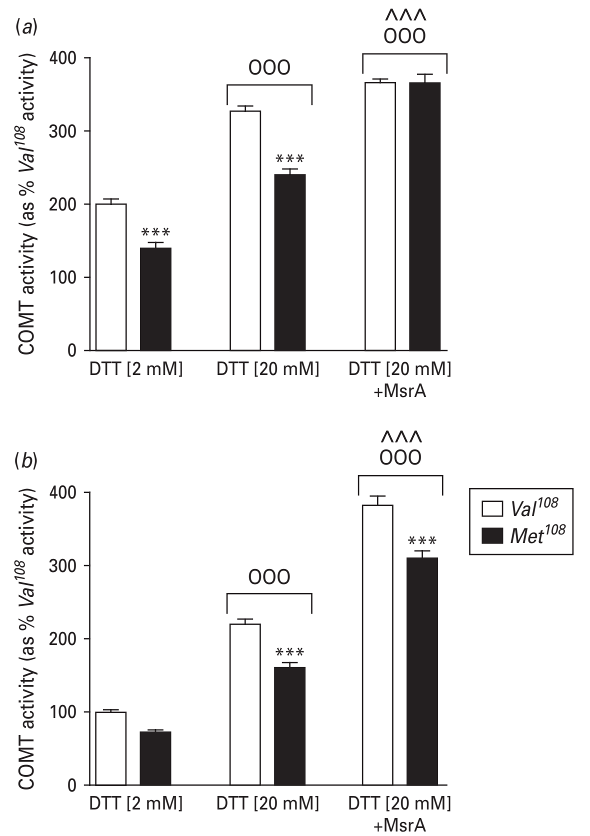

Fig. 1. Effects of dithiothreitol (DTT) and methionine sulfoxide reductase (Msr) A on the activities of catechol-*O*-methyl transferase (COMT) Val^{108} and Met^{108} variants *in vitro* under (*a*) standard conditions and (*b*) high H_2O_2 concentrations. Values are displayed as means ± S.E.M. All analyses were run by two-way analysis of variance (ANOVA), followed by Newman–Keuls test for *post-hoc* comparisons. ***, $p<0.001$ for comparisons *vs.* corresponding Val^{108} (genotype × treatment interaction); °°°, $p<0.001$ for comparison *vs.* 2 mM DTT (main treatment effect); ^^^, $p<0.01$ for comparison *vs.* 20 mM DTT (main treatment effect). Main genotype effects are not shown.

Methods

Recombinant human COMT and MsrA proteins

Expression clones for recombinant His-tag soluble human COMT proteins (S-COMT, *Val^{108}* and *Met^{108}* forms) were kindly provided by Dr Klinman (University of California, USA). The expression and purification of the recombinant COMT proteins were performed as described by Zhang and Klinman (2011). Recombinant His-tagged yeast MsrA protein was expressed and purified as previously described (Moskovitz et al., 1997). Oxidation of recombinant COMT proteins was performed by incubating the proteins with 200 mM H_2O_2 for 24 h at room temperature. Then, the residual H_2O_2 was removed by dialysis against 25 mM Tris-HCl (pH 7.4) at 4 °C.

Animals

Wild type (WT) and *MsrA* knockout (KO) mice (n=10/group) were obtained as previously reported (Moskovitz et al., 2001). Animals were housed in group cages with *ad libitum* access to food and water. The room was maintained at 22 °C, on a 12 h: 12 h light/dark cycle. Experimental procedures were in compliance with the accepted National Institute of Health guidelines (such as 'Guiding principles in the care and use of animals' (DHEW Publications, NIH, 80-23) and approved by the Animal Use Committees of the University of Kansas.

Quantification of COMT levels in brains of WT and MsrA KO mice

Mice were euthanized at 6 and 12 months of age, by CO_2 asphyxiation followed by cervical dislocation (n=5 per age group). The brains were dissected within 2 min of

Table 1. Protein sequence homology alignment between mouse and human catechol-O-methyl transferase (COMT). *Met* (M) residues are in bold and [158] *Val* of the human COMT is both in bold and underlined

Mouse	20	RHLGWGLVAIGWFEFVQQPVHNLL**M**GGTKEQRILRHVQQHAKPGDPQSVLEAIDTYCSEK	79
		RH GWGL IGW EF+QP+HNLL**M**G TKEQRIL HV QHA+PG+QSVLEAIDTYC+K	
Human	27	RHWGWGLCLIGWNEFILQPIHNLL**M**GDTKEQRILNHVLQHAEPGNAQSVLEAIDTYCEQK	86
Mouse	80	EWA**M**NVGDAKGQI**M**DAVIREYRPSLVLELGAYCGYSAVR**M**ARLLPPGARLLTMEINPDYA	139
		EWA**M**NVGD KG+I+DAVI+E++PS++LELGAYCGYSAVR**M**ARLL PGARL+T+EINPD A	
Human	87	EWA**M**NVGDKKGKI**V**DAVIQEHQPSVLLELGAYCGYSAVR**M**ARLLSPGARLITIEINPDCA	146
Mouse	140	AITQQ**M**LDFAGLQDKVSILIGASQDLIPQLKKKYDVDTLD**M**VFLDHWKDRYLPDTLLLEE	199
		AITQ+**M**+DFAG++DKV++++GASQD+IPQLKKKYDVDTLD**M**VFLDHWKDRYLPDTLLLEE	
Human	147	AITQR**M**VDFAG**<u>V</u>**KDKVTLVVGASQDIIPQLKKKYDVDTLD**M**VFLDHWKDRYLPDTLLLEE	206
Mouse	200	CGLLRKGTVLLADNVIVPGTPDFLAYVRGSSSFECTHYSSYLEY**M**KVVDGLEKAVYQGPG	259
		CGLLRKGTVLLADNVI PG PDFLA+VRGSS FECTHY S+LEY+VVDGLEKA+Y+GPG	
Human	207	CGLLRKGTVLLADNVICPGAPDFLAHVRGSSCFECTHYQSFLEYREVVDGLEKAIYKGPG	266
Mouse	260	SS	261
		S	
Human	267	SE	268

euthanasia and frozen on dry ice. The tissues were stored in −80 °C until use. Tissues were homogenized using a Teflon homogenizer in the presence of 25 mM Tris-HCl (pH 7.4) and protease inhibitors cocktail (Roche Applied Science, USA) at 4 °C. Following centrifugation at 10 000 *g* for 20 min, the supernatants were collected and their protein concentrations were assessed using a Bio-Rad (USA) assay kit. Equal protein amounts were subjected to SDS-gel electrophoresis followed by western blot analyses using anti-COMT mouse antibodies (BD Biosciences, USA) as the primary antibodies. Horseradish peroxidase (HRP)-conjugated goat anti-mouse antibodies (Santa Cruz Biotechnology, USA) were used as the secondary antibodies. Anti-ß-actin mouse antibodies (Abcam, USA) were also used on the same blot as primary antibodies (after stripping the blot) for the assessment of protein loading control. Following exposure of the blot to an X-ray film, the resulting protein band images were quantified using the Image-J program (National Institute of Health, USA).

COMT *mRNA* levels in brains of both mouse genotypes were determined by real-time polymerase chain reaction (RT-PCR) (using primers 1 418 701 and 144 918, Affymetrix, USA).

Statistical analyses

Normality and homoscedasticity of data distribution were verified by using Kolmogorov–Smirnov and Bartlett's tests. Non-parametric data were normalized by logarithmic transformation. Statistical analyses were performed using analysis of variance (ANOVA), followed by Newman–Keuls test for *post-hoc* comparisons. Significance threshold was set at 0.05. All statistical analyses were performed by STATISTICA 7 (Statsoft, USA).

Results

Effect of MetO reduction on recombinant COMT activity

To determine the combined effect of the *rs4680* polymorphism and methionine oxidation on S-COMT activity, we monitored the activity of purified His-tagged human recombinant COMT of Val^{108} (native) and Met^{108} forms. First, we analyzed the activity of the recombinant enzymes in the presence of 2 mM dithiothreitol (DTT), a reducing agent that maintains cysteine residues in their reduced form (Fig. 1*a*). Under these conditions, the-mean activity (±S.E.M) of the Met^{108} COMT variant was 69±7.72% of the native form ($p<0.001$) (Fig. 1*a*). Increasing the DTT concentration to 20 mM caused a significant increase in the activity of both COMT forms ($p<0.001$) (Fig. 1*a*), without affecting the activity gap between them (Fig. 1*a*). The addition of recombinant MsrA in the presence of 20 mM DTT further increased the activities of both forms of S-COMT ($p<0.05$), and ablated their difference in catalytic activity (Fig. 1*a*), suggesting that COMT activity is negatively affected by methionine oxidation *in vitro*, especially in the Met^{108} form. To further elucidate the possible negative effect of oxidizing COMT methionine residues (including Met^{108}) on enzyme activity, both versions of the recombinant S-COMT were exposed to high concentrations of H_2O_2 prior to monitoring COMT activity. As shown in Fig. 1*b*, COMT activity was reduced in the Met^{108} variant in comparison to the Val^{108} variant, similar to the levels observed under non-oxidizing conditions, in the presence of DTT only. However, although the addition of recombinant MsrA caused significant increases in the activities of both COMT variants ($p<0.001$) (Fig. 1*b*), there was a significant gap between the two COMT variants. These data suggest that, under severe oxidative conditions *in vitro*, the difference in activity between Val^{108} and Met^{108} forms is not fully rescued.

Genetic ablation of MsrA leads to significant reduction of COMT activity in the mouse brain

To test the relevance *in vivo* of the relation between MsrA and COMT, we measured the activity of the latter enzyme in the brains of *MsrA* KO mice, as compared with WT counterparts. The primary sequences of murine and human COMT share sequence homology for 5 methionine residues. The mouse protein contains additional methionine residues at positions 93 and 244 (Table 1), and features a leucine residue (a hydrophobic amino acid such as valine and methionine) in the position 151, homolog to 108/158 in human COMT (Table 1).

The brains of *MsrA* KO mice exhibited a marked reduction in COMT activity [Main genotype effect: $F(1,24)=175.05$, $p<0.001$] (Fig. 2*a*). The increase of DTT concentration to 20 mM produced a significant enhancement in COMT activity in both WT and KO mice (173 and 132% in comparison to WT and *MsrA* KO treated with 2 mM DTT, respectively; Main treatment effect: $F(1,24)=105.46$, $p<0.001$ in comparison with 2 mM DTT). Finally, the addition of recombinant MsrA protein to the assay reaction (in the presence of 20 mM DTT) further significantly increased COMT activity across both genotypes (*Ps*<0.001 compared with both 2 and 20 mM DTT (Fig. 2*a*). Notably, ANOVA failed to identify a significant genotype × treatment interaction, indicating that the treatment with DTT and MsrA did not abrogate the difference between genotypes.

We then analyzed whether the differences in COMT activity between WT and *MsrA* KO brains may be influenced by aging (Fig. 2*b*). Although ANOVA found significant main effects for genotype [$F(1,16)=47.40$, $p<0.001$] and age [$F(1,16)=32.64$, $p<0.001$], no significant interaction between these two effects was found, indicating that the age-dependent decline in COMT activity was not dependent on *MsrA* genotype.

To confirm that the observed difference in COMT activity between the two mouse genotypes is not attributable to a difference in COMT *mRNA* protein expression levels, COMT protein and *mRNA* levels were determined in brains of both WT and *MsrA* KO mice. Accordingly, western blot analysis and real-time PCR determination of COMT's *mRNA* levels were performed. As shown in Fig. 3, the protein levels of COMT were equivalent across both mouse genotypes. Complementary to these data, there was no significant difference in the *mRNA* levels between the two mouse genotypes (data not shown).

Discussion

The findings of this study converge in support of a regulatory role of Msr on COMT activity in the brain, and, more specifically, the PFC. We documented that MsrA

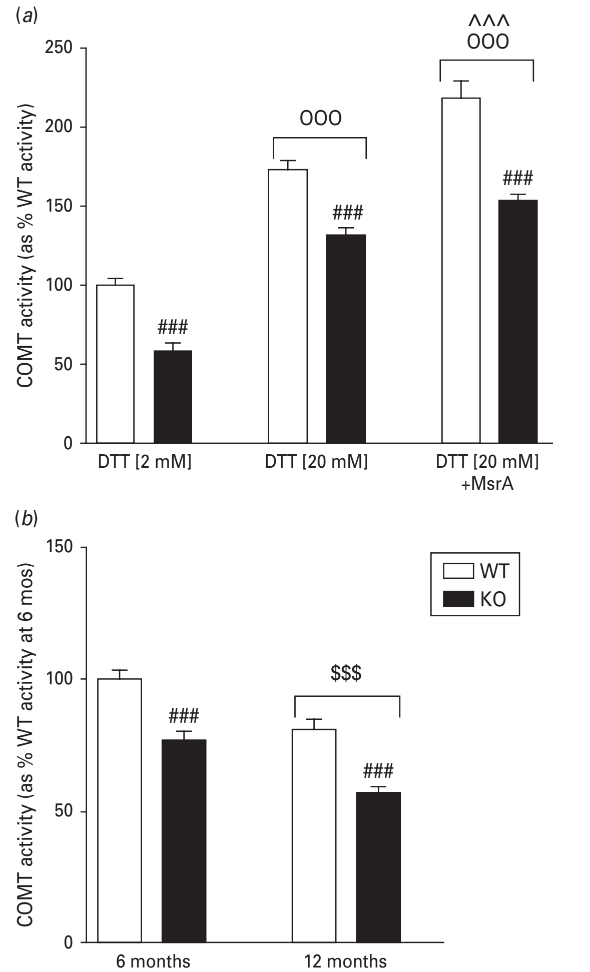

Fig. 2. (*a*) Effects of dithiothreitol (DTT) and MsrA on the brain activity of catechol-*O*-methyl transferase (COMT) in WT and *MsrA* knockout (KO) mice, and (*b*) effects of aging on COMT activity in wild-type (WT) and *MsrA* KO mice. Values are displayed as means±S.E.M. All analyses were run by two-way analysis of variance (ANOVA), followed by Newman–Keuls test for *post-hoc* comparisons. ###, $p<0.001$ for comparisons *vs.* corresponding WT group (main genotype effect); °°°, $p<0.001$ for comparison *vs.* 2 mM DTT (main treatment effect); ^^^, $p<0.01$ for comparison *vs.* 20 mM DTT (main treatment effect); $$$, $p<0.001$ for comparison *vs.* 6 months (main age effect).

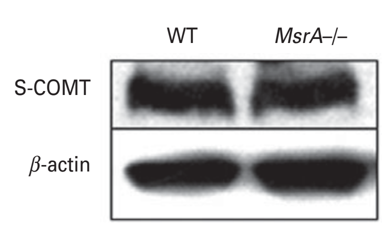

Fig. 3. Representative blots from Western blot analyses ($n=5$) showing catechol-*O*-methyl transferase (COMT) expression in comparison with β-actin expression in brain samples from wild-type (WT) and *MsrA* knockout (KO) mice.

countered the reduction of COMT activity induced by oxidation; this effect was not only limited to the *Met*[108] variant, but targeted also other methionine residues, as suggested by the ability of MsrA to enhance the catalytic activity of the *Val*[108] variant (Fig. 1). In addition, we found that in the mouse brain, COMT activity was reduced by genetic MsrA deficiency and increased by addition of recombinant MsrA; nevertheless, the latter intervention did not fully rescue the deficits of COMT activity in *MsrA* KO mice. This phenomenon suggests that in these mutants, COMT may feature post-translational modifications that may interfere with the reduction of *R*-MetO residues (Fig. 2). Supportive evidence for the involvement of post-translational modifications to the observed reduced COMT activity in *MsrA* KO mice arises from the similar expression levels of COMT's *mRNA* (data not shown) and protein (Fig. 3) in both mouse strains.

These results suggest that COMT activity may be reduced by the oxidation of its methionine residues, and complements previous evidence documenting the implication of cysteine (the other sulfur-containing amino acid) in the higher susceptibility of the *Met*[108/158] variant to oxidation (Cotton et al., 2004). Previous data have shown that methionine oxidation causes conformational changes (Berlett et al., 1996) and increases the hydrophobicity of proteins (Chao et al., 1997). Interestingly, the increase in hydrophobicity owing to MetO formation has also been shown to enhance the proteolytic susceptibility of proteins (Levine et al., 1996), suggesting that the *Met*[108/158] variant may be more vulnerable to protease-mediated degradation. Accordingly, the *Met*[108/158] variant exhibits 20% of its activity at physiological temperature; this phenomenon may be attributable to the observed reduction in the protein expression level (possibly because of its enhanced degradation rate), but not *mRNA* expression level in brain tissue (Lotta et al., 1995; Matsumoto et al., 2003a, b; Chen et al., 2004). The robust association between Msr and COMT is particularly noteworthy, in view of the primary role of COMT in the regulation of DA homeostasis in the PFC (Gogos et al., 1998; Matsumoto et al., 2003a, b). High COMT activity, such as that associated with the *Val*[108/158] variant, leads to increased DA turnover, which may result in a higher predisposition for a number of information-processing, attentional, executive and cognitive deficits in patients with psychosis, as well as healthy individuals (Kahn and Davis, 1995; Weinberger et al., 2001; Weinberger, 2002; Barnett et al., 2007; Ira et al., 2013). Furthermore, it should be noted that alterations in oxidative stress in the PFC have been associated with multiple psychiatric disorders, including schizophrenia, bipolar disorder and major depression (Michel et al., 2007; Gawryluk et al., 2011; Andreazza et al., 2013), as well as cognitive and motivational deficits in mouse models (Johnson et al., 2013). These premises highlight the potential importance of Msr as a moderator of COMT function with respect to these functional domains regulated by the PFC. Future studies are needed to test this interesting hypothesis.

Previous studies have shown that *MsrA* KO mice exhibit high levels of DA in the brain (and, particularly, in the striatum), raising the possibility that the reduction in COMT activity may be partially responsible for such changes. Nevertheless, the contribution of COMT is unlikely to fully account for the enhancement of DA levels in *MsrA* KO mice; indeed, *COMT* KO mice only featured increases in DA in the PFC, but not in the striatum (Gogos et al., 1998).

One of the main limitations of this study is that our observations were based on *S*-COMT, which is posited to play a relatively less important role in DA degradation in comparison with MB-COMT. Even with this limitation, the identification of a link between Msr and COMT activity points to the potential importance of methionine oxidation in the modulation of prefrontal activity and the pathophysiology of cognitive impairments of schizophrenia. Future clinical studies are warranted to test the implication of Msr in the function of COMT, particularly with respect to the regulation of dopamine neurotransmission and pathophysiology of schizophrenia.

Acknowledgments

This research was supported by grants from NICHD (R21HD070611, to MB) and the Hedwig Miller Fund for Aging Research (to JM). None of the institutions had any further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Statement of Interest

None.

References

Andreazza AC, Wang JF, Salmasi F, Shao L, Young LT (2013) Specific subcellular changes in oxidative stress in prefrontal cortex from patients with bipolar disorder. J Neurochem 127:552–561.

Axelrod J, Tomchick R (1958) Enzymatic *O*-methylation of epinephrine and other catechols. J Biol Chem 233:702–705.

Barnett JH, Jones PB, Robbins TW, Müller U (2007) Effects of the catechol-*O*-methyltransferase Val158Met polymorphism on executive function: a meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls. Mol Psychiatry 12:502–509.

Berlett BS, Friguet B, Yim MB, Chock PB, Stadtman ER (1996) Peroxynitrite-mediated nitration of tyrosine residues in Escherichia coli glutamine synthetase mimics adenylylation: relevance to signal transduction. Proc Natl Acad Sci U S A 93 :1776–1780.

Bilder RM, Volavka J, Czobor P, Malhotra AK, Kennedy JL, Ni X, Goldman RS, Hoptman MJ, Sheitman B, Lindenmayer JP, Citrome L, McEvoy JP, Kunz M, Chakos M, Cooper TB, Lieberman JA (2002) Neurocognitive correlates of the COMT Val(158)Met polymorphism in chronic schizophrenia. Biol Psychiatry 52:701–707.

Blasi G, Mattay VS, Bertolino A, Elvevåg B, Callicott JH, Das S, Kolachana BS, Egan MF, Goldberg TE, Weinberger DR (2005) Effect of catechol-*O*-methyltransferase val158met genotype on attentional control. J Neurosci 25:5038–5045.

Bray NJ, Buckland PR, Williams NM, Williams HJ, Norton N, Owen MJ, O'Donovan MC (2003) A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain. Am J Hum Genet 73:152–161.

Chao CC, Ma YS, Stadtman ER (1997) Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems. Proc Natl Acad Sci U S A 94:2969–2974.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-*O*-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821.

Cohen JD, Braver TS, Brown JW (2002) Computational perspectives on dopamine function in prefrontal cortex. Curr Opin Neurobiol 12:223–229.

Cotton NJ, Stoddard B, Parson WW (2004) Oxidative inhibition of human soluble catechol-*O*-methyltransferase. J Biol Chem 279:23710–23718.

Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. J Psychiatry 148:1474–1486.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A 98:6917–6922.

Ehlis AC, Reif A, Herrmann MJ, Lesch KP, Fallgatter AJ (2007) Impact of catechol-*O*-methyltransferase on prefrontal brain functioning in schizophrenia spectrum disorders. Neuropsychopharmacology 32:162–170.

Gallinat J, Bajbouj M, Sander T, Schlattmann P, Xu K, Ferro EF, Goldman D, Winterer G (2003) Association of the G1947A COMT (Val(108/158)Met) gene polymorphism with prefrontal P300 during information processing. Biol Psychiatry 54:40–48.

Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT (2011) Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. Int J Neuropsychopharmacol 14:123–130.

Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, Karayiorgou M (1998) Catechol-*O*-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. Proc Natl Acad Sci U S A 95:9991–9996.

Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, Goldman D, Weinberger D (2003) Executive subprocesses in working memory: relationship to catechol-*O*-methyltransferase Val158Met genotype and schizophrenia. Arch Gen Psychiatry 60:889–896.

Huh MM, Friedhoff AJ (1979) Multiple molecular forms of catechol-*O*-methyltransferase. Evidence for two distinct forms, and their purification and physical characterization. J Biol Chem 254:299–308.

Ira E, Zanoni M, Ruggeri M, Dazzan P, Tosato S (2013) COMT, neuropsychological function and brain structure in schizophrenia: a systematic review and neurobiological interpretation. J Psychiatry Neurosci 38:120178.

Johnson AW, Jaaro-Peled H, Shahani N, Sedlak TW, Zoubovsky S, Burruss D, Emiliani F, Sawa A, Gallagher M (2013) Cognitive and motivational deficits together with prefrontal oxidative stress in a mouse model for neuropsychiatric illness. Proc Natl Acad Sci U S A 110:12462–12467.

Kahn RS, Davis KL (1995) New developments in dopamine and schizophrenia. In: Psychopharmacology: the fourth generation of progress (Bloom FE, Kupfer DJ, eds), pp1993–1204. New York: Raven Press.

Karoum F, Chrapusta SJ, Egan MF (1994) 3-Methoxytyramine is the major metabolite of released dopamine in the rat frontal cortex: reassessment of the effects of antipsychotics on the dynamics of dopamine release and metabolism in the frontal cortex, nucleus accumbens, and striatum by a simple two pool model. J Neurochem 63:972–979.

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996) Human catechol-*O*-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243–250.

Levine RL, Mosoni L, Berlett BS, Stadtman ER (1996) Methionine residues as endogenous antioxidants in proteins. Proc Natl Acad Sci U S A 93:15036–15040.

Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melén K, Julkunen I, Taskinen J (1995) Kinetics of human soluble and membrane-bound catechol *O*-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 34:4202–4210.

Malhotra AK, Kestler LJ, Mazzanti C, Bates JA, Goldberg T, Goldman D (2002) A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. Am J Psychiatry 159:652–654.

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.

Matsumoto M, Weickert CS, Akil M, Lipska BK, Hyde TM, Herman MM, Kleinman JE, Weinberger DR (2003a) Catechol *O*-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience 116:127–137.

Matsumoto M, Weickert CS, Beltaifa S, Kolachana B, Chen J, Hyde TM, Herman MM, Weinberger DR, Kleinman JE (2003b) Catechol *O*-methyltransferase (COMT) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia. Neuropsychopharmacology 28:1521–1530.

Michel TM, Frangou S, Thiemeyer D, Camara S, Jecel J, Nara K, Brunklaus A, Zoechling R, Riederer P (2007) Evidence for oxidative stress in the frontal cortex in patients with recurrent depressive disorder – a postmortem study. Psychiatry Res 151:145–150.

Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167–202.

Moskovitz J (2005) Methionine sulfoxide reductases: ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. Biochim Biophys Acta 1703:213–219.

Moskovitz J, Berlett BS, Poston JM, Stadtman ER (1997) The yeast peptide-methionine sulfoxide reductase functions as an antioxidant *in vivo*. Proc Natl Acad Sci U S A 94:9585–9589.

Moskovitz J, Poston JM, Berlett BS, Nosworthy NJ, Szczepanowski R, Stadtman ER (2000) Identification and characterization of a putative active site for peptide methionine sulfoxide reductase (MsrA) and its substrate stereospecificity. J Biol Chem 275:14167–14172.

Moskovitz J, Bar-Noy S, Williams WM, Requena J, Berlett BS, Stadtman ER (2001) Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. Proc Natl Acad Sci U S A 98:12920–12925.

Moskovitz J, Singh VK, Requena J, Wilkinson BJ, Jayaswal RK, Stadtman ER (2002) Purification and characterization of methionine sulfoxide reductases from mouse and Staphylococcus aureus and their substrate stereospecificity. Biochem Biophys Res Commun 290:62–65.

Nieoullon A (2002) Dopamine and the regulation of cognition and attention. Prog Neurobiol 67:53–83.

Smolka MN, Schumann G, Wrase J, Grüsser SM, Flor H, Mann K, Braus DF, Goldman D, Büchel C, Heinz A (2005) Catechol-*O*-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. J Neurosci 25:836–842.

Tunbridge EM, Harrison PJ, Weinberger DR (2006) Catechol-*o*-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry 60:141–151.

Weinberger DR (2002) Schizophrenia, the prefrontal cortex, and a mechanism of genetic susceptibility. Eur Psychiatry Suppl 4:355s–362s.

Weinberger DR, Egan MF, Bertolino A, Callicott JH, Mattay VS, Lipska BK, Karayiorgou M (2001) Prefrontal neurons and the genetics of schizophrenia. Biol Psychiatry 50:825–844.

Zhang J, Klinman JP (2011) Enzymatic methyl transfer: role of an active site residue in generating active site compaction that correlates with catalytic efficiency. J Am Chem Soc 133:17134–17137.