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CATECHOL *O*-METHYLTRANSFERASE mRNA EXPRESSION IN HUMAN AND RAT BRAIN: EVIDENCE FOR A ROLE IN CORTICAL NEURONAL FUNCTION

M. MATSUMOTO,* C. SHANNON WEICKERT, M. AKIL, B. K. LIPSKA, T. M. HYDE, M. M. HERMAN, J. E. KLEINMAN AND D. R. WEINBERGER

Clinical Brain Disorders Branch, Intramural Research Program, National Institute of Mental Health, NIH, 10 Center Drive (4N312), Bethesda, MD 20892, USA

Abstract—Catechol *O*-methyltransferase (COMT) is involved in the inactivation of catecholamines, including the neurotransmitter dopamine. A Val$^{108/158}$ Met functional polymorphism of the COMT gene has been shown to affect working memory-associated frontal lobe function in humans. In the present study, *in situ* hybridization histochemistry was employed to determine the mRNA expression profile of COMT in the human prefrontal cortex, striatum and midbrain and in the rat forebrain. In both species, COMT mRNA signals were observed in large pyramidal and smaller neurons in all cortical layers of the prefrontal cortex as well as in medium and large neurons in the striatum. Levels of COMT mRNA were obviously higher in neurons than in glia. The striatum, which receives a dense dopaminergic input, expressed lower levels of COMT mRNA as compared with the prefrontal cortex. Consistent with previous protein expression data, COMT mRNA was abundant in ependymal cells lining the cerebral ventricles. In the midbrain, COMT mRNA was detected in dopaminergic neurons in both species, albeit at low levels. In the rat forebrain, dense labeling was also detected in choroid plexus and hippocampal dentate gyrus and Ammon's horn neurons. Contrary to expectations that COMT would be expressed predominantly in non-neuronal cells, the present study shows that neurons are the main cell populations expressing COMT mRNA in the prefrontal cortex and striatum. Combined with previous data about protein localization, the present results suggest that the membrane-bound isoform of COMT having a high affinity for dopamine is expressed at neuronal dendritic processes in human cortex, consistent with functional evidence that it plays an important role in dopaminergic neurotransmission. Published by Elsevier Science Ltd on behalf of IBRO.

Key words: dopamine, functional polymorphism, prefrontal cortex, striatum, midbrain schizophrenia.

Dopamine neurotransmission in the prefrontal cortex plays an important role in working memory performance by modulating synaptic inputs to prefrontal neurons (Williams and Goldman-Rakic, 1995; Goldman-Rakic, 1999; Gao et al., 2001; Seamans et al., 2001). Deficits in prefrontal working memory function are a prominent component of cognitive dysfunction in schizophrenia (Park and Holzman, 1992; Weinberger and Berman, 1996; Wexler et al., 1998; Weinberger et al., 2001), and have been linked to evidence of diminished prefrontal dopamine signaling (Weinberger et al., 1988; Akil et al., 1999; Weinberger et al., 2001). Abnormal prefrontal dopamine signaling is also implicated in working memory deficits associated with Parkinson's disease (Mattay et al., 2002b), and with normal aging (Mattay et al., 2002a).

Catechol *O*-methyltransferase (COMT) inactivates dopamine by catalyzing the transfer of a methyl group from *S*-adenosyl-L-methionine to dopamine, generating 3-methoxytyramine. COMT protein and enzyme activity are widely distributed in mammalian brain (Lundstrom et al., 1995; Mannisto and Kaakkola, 1999). In the striatum, the synaptic action of dopamine is thought to be largely terminated by neuronal uptake by abundant dopamine transporters (Giros et al., 1996). In the prefrontal cortex, however, dopamine transporters are expressed at low levels within synapses and the rate of dopamine uptake is slow (Garris et al., 1993; Sesack et al., 1998; Lewis et al., 2001; Wayment et al., 2001). Thus, in the prefrontal cortex, one might speculate that alternative mechanisms, such as degradation by COMT, might be a key process in the regulation of dopamine availability. Consistent with this notion, studies of COMT knockout mice have demonstrated that dopamine levels are increased in the prefrontal cortex of these mice, but not in the striatum, suggesting a more critical role for COMT in cortical versus subcortical sites (Gogos et al., 1998).

The expression of the COMT gene is controlled by two distinct promoters. One results in the short mRNA (1.3 kb in human and 1.6 kb in rat), which produces the soluble isoform (S-COMT). The other produces the long mRNA (1.5 kb in human and 1.9 kb in rat), yielding both soluble and membrane-bound isoforms (MB-COMT) (Mannisto and Kaakkola, 1999). A valine to methionine substitution at codon 108(soluble)/158(membrane-bound) of the COMT gene is a common polymorphism in human populations (Lotta et al., 1995; Palmatier et al., 1999), and dramatically affects COMT enzymatic activity. At normal body temperature, the met-allele variant has one-fourth the enzyme activity of the val allele (Lotta et al., 1995). Due to the potential importance of COMT in prefrontal dopamine neurotransmission, this common functional polymorphism has been studied in association with neuropsychiatric dis-

*Corresponding author. Tel: +1-301-402-2860; fax: +1-301-402-2751.
E-mail address: matsumom@intra.nimh.nih.gov, matsum_m@yamanouchi.co.jp (M. Matsumoto).

Abbreviations: BA, Brodmann's area; cDNA, complementary DNA; COMT, catechol *O*-methyltransferase; DLPFC, dorsolateral prefrontal cortex; MB-COMT, membrane-bound isoform of COMT; OCT, organic cation transporter; PCR, polymerase chain reaction; S-COMT, soluble isoform of COMT; TH, tyrosine hydroxylase.

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orders that may involve altered dopamine levels (Karayiorgou et al., 1997; Vandenbergh et al., 1997; Papolos et al., 1998; Eisenberg et al., 1999; Jones et al., 2001).

Recently, our group reported that the genotype of individuals at the COMT Val/Met polymorphism correlates with the function of prefrontal cortex (Egan et al., 2001). Normal individuals with the met/met COMT genotype, who have a less active form of COMT and should have relatively more sustained dopamine signaling, showed the best working memory performance and physiological efficiency of prefrontal cortex; individuals with the val/val genotype demonstrated the worst working memory performance and least efficient prefrontal physiology, while individuals with the val/met genotype were intermediate. The effect of the val/met genotype on working memory performance has subsequently been confirmed by several other research groups (Rosa et al., 2002; Malhotra et al., 2002). Consistent with the hypothesis of abnormal prefrontal dopamine signaling in schizophrenia, the val allele has been found to be transmitted significantly more often to probands with schizophrenia than the met al.,lele (Li et al., 1996; Kunugi et al., 1997; Egan et al., 2001).

COMT has been studied extensively since its first discovery by Axelrod and coworkers (Axelrod and Tomchick, 1958). However, its detailed anatomical expression profile in human dorsolateral prefrontal cortex (DLPFC), where dopamine plays a crucial role in enhancing working memory performance, has not been described. High levels of COMT protein have been shown to be present in non-neuronal cells, i.e. in ependymal cells of cerebral ventricles, and in choroid plexus and Bergmann glial cells, raising doubts about the neuronal expression of COMT (Kaplan et al., 1979, 1981; Karhunen et al., 1994). However, biochemical enzymatic studies have suggested that MB-COMT, which has a low K_m value (high affinity) for catecholamines, might be located in neurons (Rivett et al., 1983a,b). Electron microscopic studies also demonstrated that COMT protein is expressed in dendritic processes of neurons in the cerebral cortex and striatum (Kastner et al., 1994; Karhunen et al., 1995). Since there have been no available data on the cellular distribution of COMT mRNA in mammalian brain, it is difficult to conclude whether neurons or glia are the main cell populations expressing COMT, especially in the target areas of dopaminergic projections. In the present study, we employed *in situ* histochemical analyses to investigate COMT mRNA expression profile in human DLPFC, caudate nucleus and ventral mesencephalon. A comparative analysis of COMT mRNA in rat brain was also performed.

EXPERIMENTAL PROCEDURES

Brain tissue preparation

Human tissue specimens. Postmortem human brains were collected at the Clinical Brain Disorders Branch (NIMH) as previously described (Kleinman et al., 1995). The collection of human brain specimens was approved by the Institutional Review Board of the NIMH Intramural Research Program. Briefly, 1.5 cm coronal slabs through the hemi-sected cerebrum of each human brain were rapidly frozen in a pre-chilled dry-ice isopentane slurry bath and stored at −80°C. Brains were examined macro- and microscopically, including sections from multiple cortical areas stained with silver stain, in order to rule our neuritic pathology, cerebrovascular disease and other disorders. Cryostat sections (14 μm thick) were prepared from DLPFC (Brodmann's area 46) and striatal regions corresponding to Fig. 21 in the atlas of DeArmond et al. (1989) (two sections for each region from each subject, eight normal subjects with no diagnosed neurological or psychiatric diseases; age at death: 24–67 years, mean±S.D. 50±15 years; postmortem interval: 10–39 h, mean±S.D. 27±12 h; five females and three males). For the midbrain, anatomical levels corresponding to Fig. 57 in the atlas of Paxinos and Huang (1995) were identified in each case using Nissl-stained sections and tyrosine hydroxylase (TH) immunocytochemistry (two sections from each subject, 15 subjects with no diagnosed neurological or psychiatric diseases; age at death: 18–68 years, mean±S.D. 45±12 years; post mortem interval: 15–72 h, mean±S.D. 40±16 h; three females and 12 males).

Rat tissue specimens. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Sprague–Dawley adult male rats (270 g) were purchased from Harlan Laboratory, housed under standard laboratory conditions, and killed by decapitation one week after arrival at the NIH. Brains were removed from the skull and immediately frozen at −80°C. Coronal sections throughout the rostral-caudal extent were obtained (20 μm) and every 10th slide was used for Nissl-staining (two slices per slide from each subject, four subjects). Twenty sections per animal were chosen for COMT *in situ* hybridization study after anatomical matching based upon the Nissl-stained series from Bregma 4.2 mm to −5.6 mm according to the atlas of Paxinos and Watson (1997).

Generation of COMT riboprobe templates

T7/T3 promoter tagged COMT riboprobe templates were generated by polymerase chain reaction (PCR). To avoid non-specific amplification, full-length MB-COMT complementary DNA (cDNA) was amplified first and used as a template for a second PCR amplification for generating T7/T3 promoter tagged riboprobe templates. We amplified the same exonic region of COMT for both human and rat COMT riboprobes, from the start codon of S-COMT to just before the human Val/Met polymorphic site (321 bp), in order to make riboprobes that recognize equally both long and short transcripts. From both human and rat brain cDNA, single MB-COMT cDNA products of expected size (817 bp for human, 808 bp for rat) were amplified using primers 5'-ATGCCGGAGGCCCCGCCTCTG-3' (sense) and 5'-GTCAGGGCCCTGCTTCGCTGC-3' (antisense) for humans, and primers 5'-ATGCCGTTGGCTGCAGTCTCA-3' (sense) and 5'-GCAGGCTGAGGGATCAAGACT-3' (antisense) for rats under PCR conditions described previously (Matsumoto et al., 1995). In the next round of amplification, we used first cDNA fragments as a template and T7/T3 promoter tagged primers 5'-CAGAGATGCATAATACGACTCACTATAGGGAGAATGGGTGACACCAAGGAGCAG-3' (sense, artificial T7 promoter sequence is underlined) and 5'-CCAAGCCTTCATTAACCCTCACTAAAGGGAGAGCCAGCGAAATCCACCATCCG-3' (antisense, artificial T3 promoter sequence is underlined) for humans and primers 5'-CAGAGATGCATAATACGACTCACTATAGGGAGAATGGGTGACACAAAGGAGCAG-3' (sense, T7 promoter) and 5'-CCAAGCCTTCATTAACCCTCACTAAAGGGAGAGCCTGCAAAGTTCAGCATTTG-3' (antisense, T3 promoter) for rats. Single bands of expected size of T7/T3 promoter tagged fragments (386 bp including T7/T3 promoters) were amplified from both human and rat and confirmed to have the identical sequence as the COMT previously reported (Bertocci et al., 1991; Tenhunen et al., 1993).

Northern blot analysis

Northern blot analysis was used to confirm the specificity of the human COMT riboprobe for COMT transcripts in human brain RNA. A ^{32}P-labeled antisense riboprobe with specific activity of 1×10^9 cpm/μg was synthesized using T3 RNA polymerase from the T7/T3 promoter tagged COMT riboprobe templates. A MTN RNA blot containing cortical regions from adult human brain (Clontech, Palo Alto, CA, USA) was pre-hybridized (1 h) and hybridized (1 h) with 2×10^6 cpm/ml of riboprobe in ExpressHyb buffer (Clontech) at 68°C according to the manufacture's protocol. The blot was rinsed three times in 2× SSC/0.1% SDS at room temperature, and twice in 0.1× SSC/0.1% SDS at 68°C (30 min). The blot was exposed to X-OMAT film (Kodak, Rochester, NY, USA) overnight with an intensifying screen.

In situ hybridization

Fixation, acetylation, delipidation and dehydration of the slices were performed according to standard protocols (Whitfield et al., 1990). ^{35}S-UTP labeled riboprobes for COMT with specific activity of 1×10^9 cpm/μg were synthesized using T3 (for antisense) and T7 (for sense) RNA polymerase from the T7/T3 promoter tagged COMT riboprobe templates. ^{35}S-UTP labeled riboprobes for human tyrosine hydroxylase (human midbrain sections only) were synthesized as previously described (Joh et al., 1998). Two hundred microliters of hybridization buffer for human sections and 50 μl of hybridization buffer for rat sections containing the ^{35}S-UTP labeled species-specific COMT riboprobes (5 ng/ml) were added to each section and hybridization was allowed to occur at 55°C overnight in humidified chambers. After hybridization, slides were treated with RNase A and then washed under high stringency conditions (0.2× SSC, 55°C for 1 h and 0.2× SSC, 60°C for 1 h). After dehydration, hybridized slides, along with ^{14}C standards (American Radiolabeled Chemicals, Inc., St Louis, MO, USA) were exposed to Kodak BioMax film for 1 week. Slides were dipped in NT-B2 emulsion (Kodak), stored in light-tight boxes in the dark for 2 months (human slides) or 1 month (rat slides) and then developed in D-19 developer (Kodak), dehydrated and lightly stained with a Nissl counterstain. For the human slides, the sections containing the DLPFC and caudate nucleus were processed together, so that direct quantitative comparisons could be made. Human tissue sections from the ventral mesencephalon were used for assessment of COMT and TH mRNA expression profiles in separate experiments. All sections of the rat brain were processed in one experiment.

Image analysis

Optical density measurements of the X-ray film were performed with the aid of the NIH Image (Rasband, NIH). For human DLPFC, with the aid of a microscope, the boundary of Brodmann's area 46 (BA 46) was delineated on Nissl-stained sections, applying the criteria described by Rajkowska and Goldman-Rakic (1995). Sampling was done in BA 46 where the cortical layers were aligned parallel to the pial surface. A rectangular box (2.1 mm^2) was placed along the middle frontal sulcus and the optical density for COMT mRNA in the gray matter was obtained by taking the average of 6 such boxes from the 2 tissue sections per subject. For the caudate nucleus, a circular area (1.7 mm^2) was placed at the central part of the caudate nucleus and the optical density was obtained by taking the average of six such circles from the two tissue sections per subject. For the ventral mesencephalon, the distribution patterns of COMT and TH mRNA signals from adjacent sections on the film were compared visually. Identification of rat brain regions was based upon the atlas of Paxinos and Watson (1997). For the rat prefrontal cortex, optical density for COMT mRNA was obtained by averaging the whole infralimbic and prelimbic cortical areas (1.2 mm^2) at the level of Bregma 2.7-3.2 mm from both hemispheres from two slices per subject. For the rat striatum, optical density was obtained by taking the average of a circular area (1.2 mm^2) placed at the central part of the striatum at the level of Bregma 1.5–1.7 mm from both hemispheres from two slices per subject. Optical densities normalized for sampled area (μCi/g) per region were averaged across slices per subject. Regional comparisons were performed by paired *t*-test. For characterization of cellular labeling, slides were examined by low-power dark-field and high-power bright-field microscopy. Images of silver grains from these slides were captured using a video camera mounted on a microscope Axiophot (ZEISS, Gottingen, Germany).

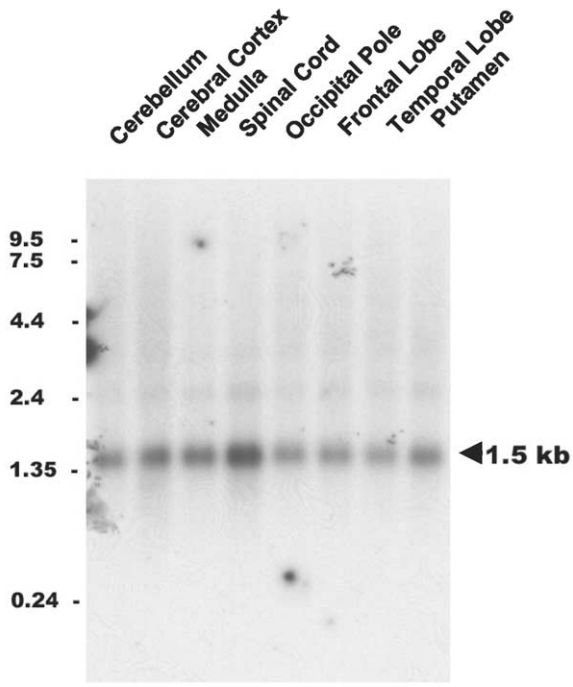

Fig. 1. Northern blot analysis of COMT mRNA in human brain regions. A RNA blot containing 2 μg of poly A+ RNA from various adult brain regions was hybridized with ^{32}P-labeled antisense riboprobes made from T7/T3 promoter tagged COMT cDNA template. Positions of molecular size standards are indicated at left. One major band (1.5 kb) was detected in both cortical and subcortical regions.

RESULTS

In situ analysis of COMT mRNA in the human DLPFC, caudate nucleus and ventral mesencephalon

In order to confirm the specificity of the COMT riboprobe, we performed Northern blot analysis of human brain regions with the ^{32}P-labeled antisense riboprobe. Although this riboprobe can recognize both long and short transcripts of COMT (see Experimental Procedures), only one major band at 1.5 kb (long form) was detected. COMT 1.5 kb transcripts were clearly detected in all human brain regions including cerebral cortices (Fig. 1).

Hybridization of tissue sections with the ^{35}S-labeled antisense riboprobe generated fairly robust signal (Fig. 2), whereas hybridization of tissue with the sense riboprobe showed no obvious background signal (data not shown).

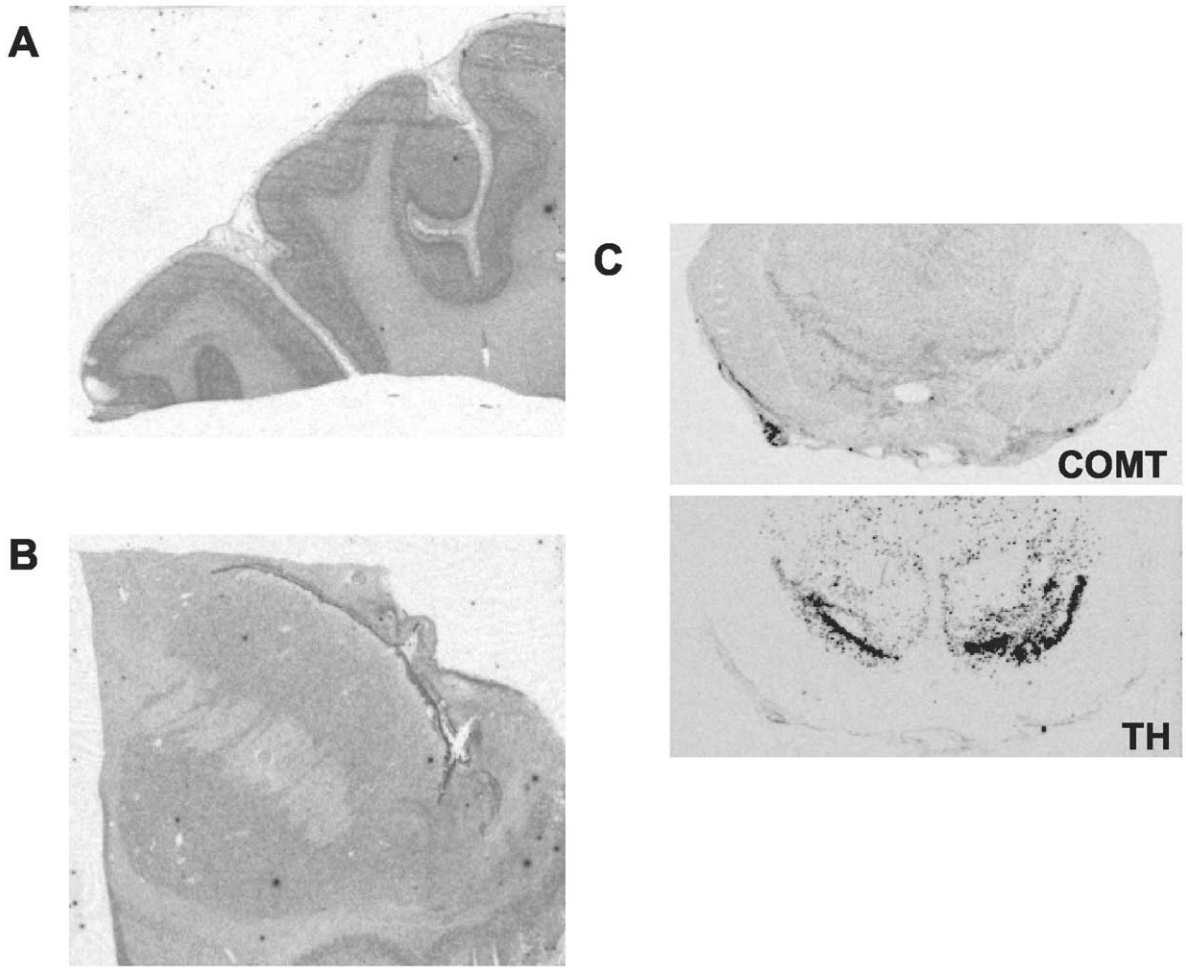

Fig. 2. X-ray film autoradiogram images of *in situ* analysis of COMT mRNA in human brain. Representative X-ray film autoradiogram images of COMT mRNA from coronal sections of human DLPFC (A) and caudate nucleus (B) (from the same subject) are shown. Representative images of COMT mRNA and TH mRNA from human midbrain (adjacent sections from the same subject) are shown (C).

The distribution pattern of the COMT mRNA hybridization signal was similar from one brain to another but its overall intensity was somewhat variable among subjects. Representative X-ray film autoradiograms of DLPFC, caudate nucleus (from the same subject) and ventral mesencephalon sections (presented with the tyrosine hydroxylase mRNA signals from adjacent sections from the same subject) are shown in Fig. 2. In DLPFC, neocortical layers II, IV and VI showed the most intense COMT mRNA signals on X-ray film. Detected signals were relatively high in large pyramidal neurons compared with smaller neurons. In all layers examined, silver grains were more numerous overlying large lightly stained neurons than small non-neuronal glial profiles where signal was typically at background levels (Fig. 3A). In the caudate nucleus, COMT mRNA was also mainly detected in neurons of medium size, albeit at low levels, and in large (presumptively cholinergic) neurons at relatively high levels (Fig. 3B). Again, the COMT mRNA signal was rarely detected in glial cells. When we compared the intensities of COMT hybridization signal on X-ray film between gray matter areas of human DLPFC and caudate nucleus, the mean optical density in the DLPFC was significantly greater (mean$\pm$S.D.$=0.107\pm0.017$ μCi/g in DLPFC versus 0.069 ± 0.012 μCi/g in caudate nucleus, $P<0.0001$). In the sections containing the caudate nucleus, intense COMT mRNA expression was detected in ependymal cells lining the lateral ventricle (Fig. 3B). In the ventral mesencephalon, low levels of COMT mRNA signals were detected in dopaminergic neurons in the substantia nigra pars compacta and the ventral tegmental area (Fig. 3C), though the expression pattern appeared to be similar to that of tyrosine hydroxylase (Fig. 2C). Because of the low mRNA expression of COMT in all dopaminergic neurons, it was difficult to discern differences between dopaminergic cell groups.

In situ analysis of COMT mRNA in rat forebrain

Antisense riboprobes to COMT generated intense signals when hybridized to the rat forebrain slices (Fig. 4). There was no obvious hybridization signal when rat tissue was treated with the sense riboprobe (data not shown). In the rat frontal cortex, COMT mRNA was detected in neurons in all cortical layers. In all layers examined, higher levels of COMT mRNA were detected in neurons than in non-neu-

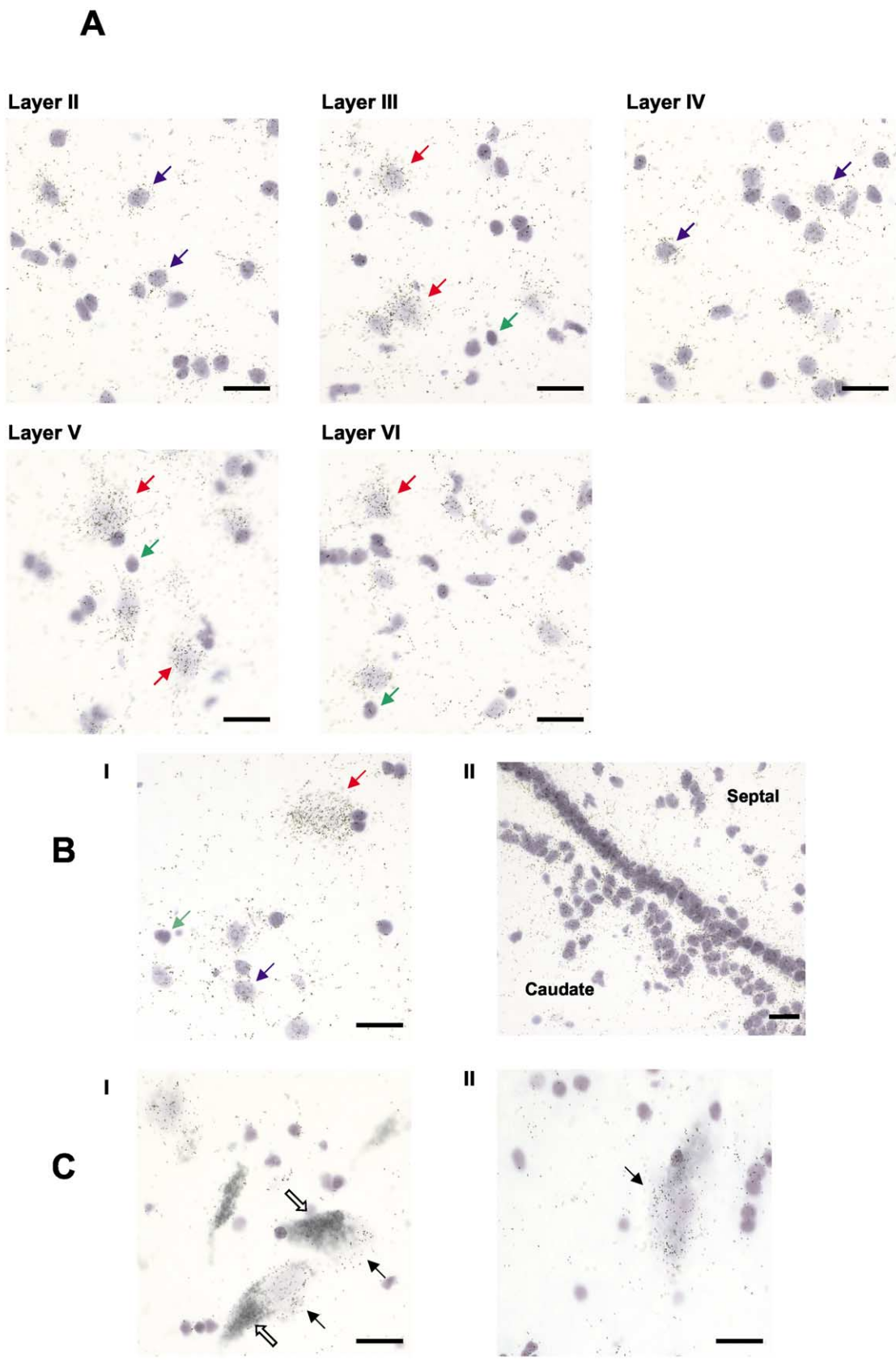

Fig. 3. (*Caption overleaf*).

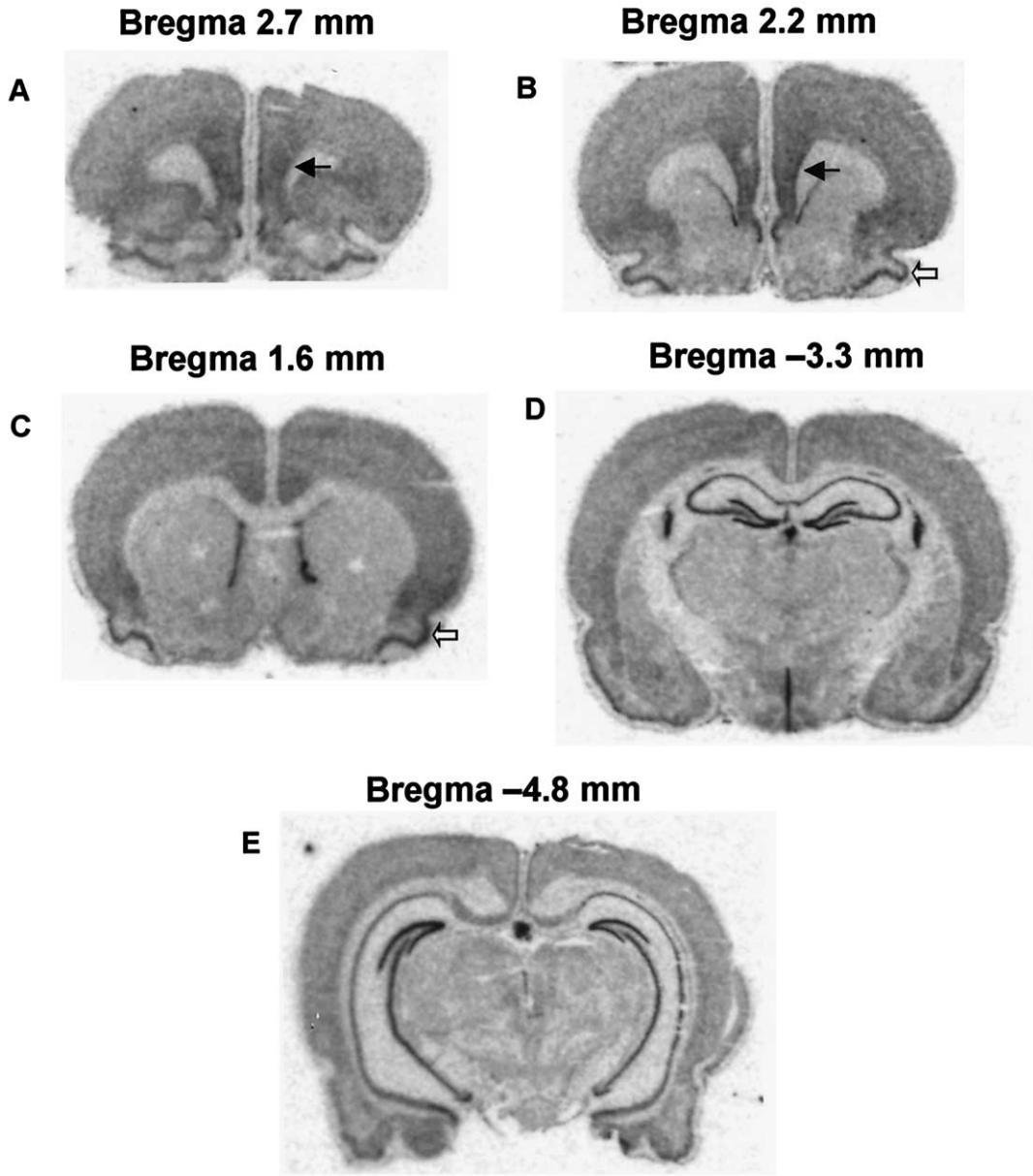

Fig. 4. X-ray film autoradiogram images of *in situ* analysis of COMT mRNA in rat brain. Representative X-ray film autoradiogram images of COMT mRNA from coronal sections of rat forebrain are shown. Intense COMT mRNA signals can be seen on ependymal cells lining the lateral (B, C, D) and third (D) ventricles, in the choroid plexus of the lateral (D) and dorsal third (D, E) ventricles, and in the hippocampus (D, E). Note that relatively high levels of COMT mRNA are present in the prefrontal cortex (infralimbic and prelimbic cortices, arrows in A and B) and piriform cortex (open arrows in B and C) compared with the primary motor and somatosensory cortices (A, B, C).

ronal glial cells (Fig. 5A). In the cortical areas, COMT mRNA was detected at relatively higher densities on X-ray film in prefrontal and piriform cortices compared with the motor and somatosensory cortices (Fig. 4). In the striatum, COMT mRNA was also detected mainly in neurons (Fig. 5B), however, its density on X-ray film was significantly lower than that

Fig. 3. High power bright field images of *in situ* analysis of COMT mRNA in human brain. Photomicrographs showing COMT mRNA expression (A) in the DLPFC. Note that large pyramidal neurons contain COMT silver grains at high levels (red arrows) and smaller sized neurons contain relatively lower levels of silver grains (blue arrows). Glial cells identifiable by small darkly Nissl-stained nuclei have silver grains at background levels (green arrows). (B) In the caudate nucleus (I), and ependyma (II) from the striatal section. Note that large cholinergic neurons contain COMT silver grains at high levels (red arrow in I) and medium-sized neurons contain low levels of silver grains (blue arrow in I). Glial cells identifiable by small darkly Nissl-stained nuclei have silver grains at background levels (green arrow in I). (C) In the substantia nigra compacta (I), and ventral tegmental area (II) from the ventral mesencephalon. Note that solid arrows indicate dopaminergic neurons containing COMT silver grains. Open arrows in I indicate dark-colored neuromelanin in the dopaminergic neurons distinguishable from small black dots of silver grains. All photomicrographs in (A) and (B) are from the same subject. (Scale bar=20 μM).

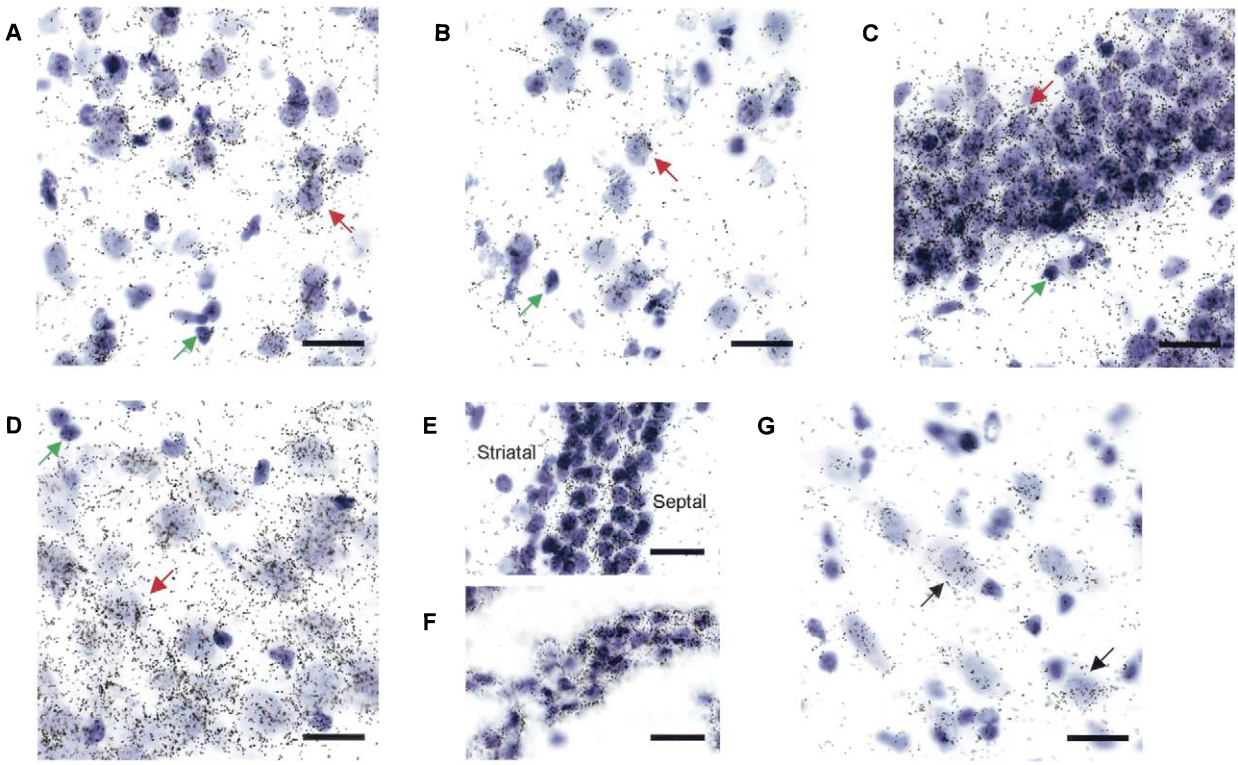

Fig. 5. High power bright field images of *in situ* analysis of COMT mRNA in rat brain. Photomicrographs showing COMT mRNA expression in the prefrontal cortex deep layer (A), striatum (B), hippocampal dentate gyrus (C), hippocampal CA3 (D), ependyma (E), choroid plexus (F), and substantia nigra pars compacta (G). Red arrows in A–D indicate neurons containing COMT silver grains (A: pyramidal neurons, B: medium-sized neurons, C: granular cells, D: pyramidal neurons). Green arrows in A–D point to glial cells (silver grains are at background levels). Solid arrows in G indicate dopaminergic neurons containing COMT silver grains. (Scale bar=20 μm).

of the prefrontal cortex (mean±S.D.=0.122±0.012 μCi/g in striatum versus 0.218±0.029 μCi/g in prefrontal cortex, $P<0.01$). In the rat forebrain sections, a dense COMT hybridization signal was detected in ependymal cells lining the cerebral ventricles, choroid plexus and hippocampus (Figs. 4, 5C–F). In the hippocampal formation, granule cell neurons in dentate gyrus and pyramidal neurons in all CA regions densely expressed COMT mRNA. In the mesencephalon, COMT mRNA was detected at low levels in dopaminergic neurons in the substantia nigra pars compacta and the ventral tegmental area (Fig. 5G).

DISCUSSION

In the present study, we describe aspects of the mRNA expression profile of COMT in the human and rat brain with a particular emphasis on regions receiving dopaminergic input or containing dopaminergic neurons. Our results suggest that COMT is particularly important in the postsynaptic metabolism of catecholamines in cortex, primarily mediated by neurons and not glia.

COMT mRNA distribution in the rat and human brain

The regional distribution pattern of COMT mRNA in the present study is consistent with the localization of COMT protein reported previously (Kaplan et al., 1979; Karhunen et al., 1994). In the rat forebrain, the most intense COMT mRNA signal was detected in ependymal cells lining the cerebral ventricles and in the choroid plexus. By immunohistochemical analysis, these two regions have been reported to have COMT proteins at the highest levels in rat brain (Kaplan et al., 1979; Karhunen et al., 1994). In the human striatal section, high levels of COMT mRNA signal were also detected in ependymal cells. In addition, strong COMT mRNA hybridization signals were detected in rat hippocampal dentate gyrus and the CA regions in the present study, although COMT protein expression in hippocampus has not been reported by previous investigators. However, our data are consistent with previous biochemical data that showed high COMT enzymatic activities in rat hippocampus (Saavedra et al., 1976). In both humans and rats, we detected higher densities of COMT mRNA in the prefrontal cortex than in the striatum. Again, our results are consistent with previous biochemical data (Saavedra et al., 1976) showing lower COMT enzyme activity in the rodent striatum than in the frontal cortex. Because COMT mRNA signals were detected at different levels among neuronal cell types even in the same brain area, e.g. relatively higher signals in large pyramidal neurons compared with smaller neurons possibly due to the large cell volume of pyramidal cells in the human DLPFC, further characterization of COMT expression in different type of neurons would be necessary to clarify the difference of COMT mRNA density between the prefrontal cor-

tex and striatum. In both species, COMT mRNA signals were detected in dopaminergic neurons in the substantia nigra pars compacta and ventral tegmental area, albeit at low levels. Previous biochemical and immunohistochemical data showed that COMT protein was not detected at the presynaptic terminals of dopaminergic neurons in the striatum (Kaakkola et al., 1987; Kastner et al., 1994; Karhunen et al., 1995; Lundstrom et al., 1995). It is possible that COMT protein might be located at cell bodies and/or dendritic processes of dopaminergic neurons in the midbrain, but not their presynaptic nerve terminals in the striatum. Consistent with this interpretation, an immunohistochemical study by Kastner et al. (1994) showed that low levels of COMT protein were detected in the cell bodies of some dopaminergic neurons in human midbrain, but not in dopaminergic nerve terminals in the striatum. Although Kastner et al. (1994) detected the presence of COMT protein predominantly in dopaminergic cell groups in the ventral tegmental area and substantia nigra pars lateralis, the low levels of COMT mRNA expression in the ventral mesencephalon may have limited our ability to discern differences in levels of expression between the cell groups.

COMT mRNA expression in neurons

In the present study, COMT mRNA was detected at much higher levels in neurons than in non-neuronal glial cells in prefrontal cortex and striatum in both human and rat. In addition, granular and pyramidal neurons in the dentate gyrus and CA regions in rat hippocampus showed abundant expression of COMT mRNA. This neuronal expression profile is quite different from prior general predictions, namely that COMT would be expressed mainly in non-neuronal cells in the brain (Kaplan et al., 1979; Lundstrom et al., 1995; Mannisto and Kaakkola, 1999). On the contrary, immunoelectron microscopic studies have reported that COMT protein was present in dendritic processes of neurons (Kastner et al., 1994; Karhunen et al., 1995). Previous biochemical studies also suggested that MB-COMT, which has a low K_m value (high affinity) for catecholamines, might be located in neurons (Rivett et al., 1983a,b). Because dense COMT proteins were detected in specific non-neuronal cells in brain, i.e. in ependymal cells, choroid plexus cells and Bergmann glial cells, it is indeed possible that previous studies have overestimated non-neuronal expression and underestimated neuronal expression of COMT. Also, because COMT proteins were detected predominantly in the neuropil but not in neuronal or glial cell bodies in the cerebral cortex and striatum in previous light microscopic immunohistochemical studies (Kaplan et al., 1979; Karhunen et al., 1994; Lundstrom et al., 1995), it was difficult to judge whether those proteins were originally synthesized in neurons or in glial cells. The present data suggest that COMT proteins in the neuropil, i.e. possibly in dendritic processes, spines and axons, would be predominantly neuronal in origin, particularly in the prefrontal cortex where we have found much higher levels of COMT mRNA in neurons than in glial cells.

Although the riboprobes used in the present study are able to equally recognize both short and long isoforms of COMT mRNA, only the long isoform of 1.5 kb was detected by Northern blot analysis in human brain regions, including cerebral cortical areas. This pattern of hybridization exactly matches previous data by Tenhunen et al. (1994) and Hong et al. (1998), showing the presence of only 1.5 kb mRNA in human brain, despite the fact that their probe and our riboprobe were made from different portions of the COMT mRNA. In rat brain, the long (1.9 kb) mRNA was also detected as the predominant form (Tenhunen and Ulmanen, 1993). Therefore, most of mRNA signals detected in the present *in situ* study likely represent the long isoform of COMT transcript. Although it has been shown that the long mRNA produces S-COMT as well as MB-COMT by the leaky scanning mechanism of translation-initiation, Tenhunen et al. (1994) have reported that the first AUG initiation codon in the human long COMT mRNA is situated in a more favorable location, enabling most of the ribosome to initiate translation at this site. Consistent with these data, MB-COMT protein was detected as a predominant form in human cerebral cortices (70% of total, Tenhunen et al., 1994), whereas S-COMT was detected as a predominant form in rat brain telencephalon (70% of total, Tenhunen and Ulmanen, 1993).

The precise subcellular localization of MB-COMT remains an unanswered question. As postulated earlier, if MB-COMT is present on the outer surface of the plasma membrane, COMT can directly access intrasynaptic dopamine for *O*-methylation. However, as pointed out by Roth (1992), MB-COMT activity would be greatly diminished by the high concentrations of extracellular Ca^{2+}. Recent protein experiments (Ulmanen and Lundstom, 1991, 1997) and the distribution of charged amino acids flanking the anchor domain (Hartmann et al., 1989) suggest that MB-COMT is oriented on the cytoplasmic side of the rough endoplasmic reticulum. When MB-COMT protein was translocated to the plasma membrane, this postulated intracellular orientation would result in its localization to the inner surface. In this case, even if we assumed that MB-COMT is located at the dendritic membranes postsynaptically in prefrontal neurons, some transport mechanism would be needed for the metabolism of dopamine by MB-COMT. Since high affinity dopamine transporters are rarely expressed in prefrontal neurons intrasynaptically, recent reports regarding organic cation transporters (OCTs), which are capable of transporting dopamine, are potentially important. Busch et al. (1998) and Wu et al. (1998) have shown that the OCTs, OCT2 and OCT3, are expressed in neurons in human and rat brain, including cerebral cortex and hippocampus. They demonstrated that OCT2 and OCT3 are able to transport dopamine, albeit with relatively low affinity, and have postulated that the OCTs represent a background transporter for monoamine neurotransmitters. Given the data that OCTs translocate cations in both directions, it is interesting to speculate that MB-COMT and OCTs might regulate active dopamine concentration at the synaptic cleft. In this scheme, because

MB-COMT enzymatic activity is inhibited by Ca^{2+} and OCTs activities are sensitive to the membrane potential (Roth, 1992; Busch et al., 1998; Wu et al., 1998), synaptic dopamine concentration might be regulated in part by postsynaptic neuronal activity in the prefrontal cortex.

Our present data have confirmed, at least partly, the previous work showing that low levels of COMT protein are present in human dopaminergic neurons (Kastner et al., 1994). As discussed above, COMT protein is likely to be located on the soma and/or dendritic processes of dopaminergic neurons. Kastner et al. (1994) have postulated that COMT might actively protect dopaminergic neurons against cytotoxic free radicals generated by oxidative metabolism of dopamine. Dendritic release of dopamine from dopaminergic neurons in substantia nigra is well-documented and is believed to be involved in the communication between dopaminergic neurons within the pars compacta and/or between dopaminergic neurons and GABAergic neurons in the pars reticulata (Cheramy et al., 1981; Falkenburger et al., 2001). COMT expressed in dopaminergic neuronal soma and dendrites might degrade released dopamine from dendrites and/or regulate dopamine release from dendrites.

Abundant COMT mRNA expression in granular and pyramidal neurons in the rat hippocampal formation suggests a relevant role in hippocampal information processing as well. Considering the low levels of dopamine but high levels of norepinephrine in the hippocampal formation (Diop et al., 1988), one might speculate that COMT plays a more pivotal role in regulating norepinephrine rather than dopamine signaling in the hippocampus. However, it is worth noting that dopamine D1 and D5 receptors are expressed in the hippocampus, especially in pyramidal cells (Bergson et al., 1995; Khan et al., 2000; Montague et al., 2001). Thus, COMT might also regulate dopamine signaling by the mechanism postulated above, because OCTs have been reported to be expressed in hippocampal neurons at high levels (Busch et al., 1998; Wu et al., 1998).

Role of COMT in dopaminergic neurotransmission in prefrontal cortex

The neuronal expression profile of COMT in human DLPFC presented in this study is potentially important in terms of dopamine neurotransmission in this brain region. COMT has been considered especially relevant in the termination of the dopamine signal in the prefrontal cortex, because high affinity dopamine transporters are expressed in low abundance and apparently not within synapses in this region (Sesack et al., 1998; Lewis et al., 2001). The importance of COMT in the prefrontal cortical dopamine metabolism is also supported by previous animal studies. Karoum et al. (1994) reported that *O*-methylation is a prominent step in the clearance of dopamine in the prefrontal cortex but not in the striatum. A study of COMT knockout mice found that dopamine levels were increased only in the prefrontal cortex and not in the striatum (Gogos et al., 1998). Interestingly, changes in prefrontal norepinephrine concentrations, another COMT substrate, were not altered in COMT knockout mice, consistent with the interpretation that norepinephrine transporters, which are abundantly expressed in prefrontal cortex (Moron et al., 2002), are rate limiting in norepinephrine signaling inactivation. Further evidence for the relevance of COMT in dopamine signaling in prefrontal cortex is our observation that COMT mRNA is expressed at higher levels in prefrontal cortex than in striatum in both humans and rats. This regional distribution pattern is especially intriguing, considering that the striatum has a much greater tissue concentration of dopamine and a much more dense dopamine innervation (Diop et al., 1988). Additionally, in rat cerebral cortex, COMT mRNA is unevenly distributed. Relatively higher mRNA densities were detected in prefrontal and piriform areas compared with primary motor and somatosensory areas. This cortical distribution profile is similar to cortical dopamine content and to D1 receptor densities but not to the distribution of norepinephrine (Diop et al., 1988).

As discussed above, the present data suggest that MB-COMT is the predominant form expressed in human DLPFC neurons. The K_m value of MB-COMT for dopamine is 10–100 times lower (higher affinity) than that of S-COMT (Rivett and Roth, 1982; Lotta et al., 1995). This indicates that MB-COMT would be more relevant at the low physiological concentrations of dopamine in mammalian brain (Roth, 1992), especially in the prefrontal cortex where dopamine concentration and the dopamine transporter densities are much lower that in the striatum.

In summary, we have found that the long form of COMT mRNA, which produces MB-COMT having a high affinity for dopamine, was expressed predominantly in neurons and in relatively high abundance in human DLPFC. Molecular genetics studies have revealed associations between the COMT Val $^{108/158}$Met functional polymorphism and various psychiatric disorders, including attention deficit hyperactivity disorder (Eisenberg et al., 1999), bipolar disorder (Papolos et al., 1998), substance abuse (Vandenbergh et al., 1997) and schizophrenia (Kunugi et al., 1997; Li et al., 1996, 2000; Egan et al., 2001). It is likely that all of these syndromes represent a spectrum of emergent clinical phenomena related to prefrontal functional variation (Weinberger et al., 2001). Because optimal levels of dopamine D1/D5 stimulation is needed to optimize working memory function in the prefrontal cortex (Williams and Goldman-Rakic, 1995), the effect of COMT activity on dopamine signaling in this region is likely to have an observable behavioral phenotype. Our present data add to the evidence that COMT is potentially directly involved in the regulation of synaptic dopamine concentration at the dendritic processes of prefrontal neurons.

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