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COMT Val$^{108/158}$Met genotype affects the mu-opioid receptor system in the human brain: Evidence from ligand-binding, G-protein activation and preproenkephalin mRNA expression

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Recent data from [^{11}C]carfentanil ligand-PET indicate that in the human brain, the availability of mu-opioid (MOP) receptor binding sites is affected by the Val$^{108/158}$Met polymorphism of the *catechol-O-methyltransferase (COMT)* gene. This prompted us to validate the impact of *COMT* Val$^{108/158}$Met on MOP receptors in human post-mortem brain. [^{3}H]DAMGO receptor autoradiography was performed in frontal cortex, basal ganglia, thalamus and cerebellum (8 Met/Met, 6 Met/Val, 3 Val/Val). With respect to genotype, numbers of MOP binding sites in *COMT* Met$^{108/158}$ homozygous and Val$^{108/158}$Met heterozygous cases were higher than in Val$^{108/158}$ homozygous. Differences were significant in the caudate nucleus (Val/Met vs. Val/Val), nucleus accumbens (Val/Met vs. Val/Val) and the mediodorsal nucleus of the thalamus (Met/Met vs. Val/Val). In the thalamus, this was corroborated by DAMGO-stimulated [^{35}S]GTPγS autoradiography. Moreover, stepwise multiple regression taking into account various covariables allowed to confirm the *COMT* genotype as the most predictive factor in this structure.

As a mechanism how COMT might exert its action on MOP receptors, it has been suggested that at least in striatopallidal circuits *COMT* Val$^{108/158}$Met impacts on enkephalin, which is capable of reciprocally regulating MOP receptor expression. Thus, we assessed preproenkephalin mRNA by in situ hybridization. In the striatum, mRNA levels were significantly higher in *COMT* Met$^{108/158}$ homozygous cases indicating that MOP binding sites and enkephalin are regulated in parallel. Moreover, the transcript was not detectable in the thalamus. Thus, mechanisms other than an enkephalin-dependent receptor turnover must be responsible for *COMT*-related differences in MOP binding site availability in the human brain.

Keywords: Ligand-binding; G-protein activation; Preproenkephalin; Opioid receptor system; Gene polymorphism

Introduction

Although the search for genetic variation of human behavior has attracted increasing efforts, so far only very few single genes have turned out to convey strong effects to CNS function. One candidate which has been extensively studied is the gene for catechol-*O*-methytransferase (*COMT*). COMT and monoamine-oxidase are the pivotal enzymes of catecholamine catabolism. The *COMT* gene encodes two isoenzymes: a shorter soluble one (S-COMT) and a longer enzyme, which is membrane-bound (MB-COMT). The human *COMT* gene (but not the nonhuman primate or rodent) contains a polymorphism which results in a valine (Val) to methionine (Met) substitution at position 158 (MB-COMT) resp. 108 (S-COMT) of the protein. This substitution is functional in the way that it conveys differing enzymatic activity to the protein. In the liver, COMT activity is almost four times higher in Val$^{108/158}$ homozygous than in Met$^{108/158}$ homozygous individuals and displays an almost linear gene dose effect (Lachman et al., 1996; Shield et al., 2004). Only recently, this impact of the *COMT* Val$^{108/158}$Met polymorphism on the enzymatic activity has been confirmed for MB-COMT isolated from human post-mortem dorsolateral prefrontal cortex (Chen et al., 2004). Thus, it can be assumed that the *COMT* genotype influences the homeostasis of catecholamines in the human brain. In line with this, Akil et al. (2003) demonstrated that in the mesencephalon, the amount of mRNA for tyrosine hydroxylase,

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the enzyme synthesizing dopamine, is related to the *COMT* genotype. Functionally, the *COMT* genotype is known to be associated with variations in cognitive performance in healthy volunteers and is very likely of importance for psychiatric diseases (Bilder et al., 2002; Egan et al., 2001; Malhotra et al., 2002).

A recent study indicates that the *COMT* Val$^{108/158}$Met polymorphism may have functional implications beyond the catecholamine system. By means of ligand-PET with [^{11}C]carfentanil, a mu-opioid (MOP) receptor ligand, Zubieta et al. (2003) were able to demonstrate that the *COMT* genotype impacts on the amount of binding sites of the G-protein coupled opioid receptors. In this study, the basal binding potential of [^{11}C]carfentanil as a measure of the basal expression of binding sites significantly differed between genotypes in the anterior thalamus and pulvinar. Binding potential was highest in *COMT* Met$^{108/158}$ homozygous, lower in heterozygous and lowest in *COMT* Val$^{108/158}$ homozygous individuals. Possible genotype effects were observed in the nucleus accumbens and the ventral pallidum.

Since in ligand-PET tracers are applied in low concentrations, the method is sensitive to the occupation of receptors by endogenous receptor ligands. This allows functional receptor activation studies in vivo, which is recognized as a major advantage of the technique. Employing such a receptor activation paradigm, the authors presented in vivo data indicating that following a painful stimulus the release of endogenous opioidergic ligands in the thalamus and the basal ganglia is less in *COMT* Met$^{108/158}$ homozygous healthy volunteers than in *COMT* Val$^{108/158}$Met heterozygous.

However, the sensitivity of ligand-PET to endogenous ligands may turn out to be a drawback when basal receptor expression patterns are compared, since differences in binding patterns between experimental groups may derive from true differences in receptor binding site numbers, but as well from differences in the occupation of binding sites by tonically liberated endogenous ligands. This prompted us to investigate whether the impact of the *COMT* Val$^{108/158}$Met polymorphism indeed influences MOP receptor binding site availability in terms of binding site expression. To do so, we employed ex vivo receptor binding autoradiography in post-mortem tissue. This technique allows to apply the radioligand in saturating concentrations to unmask most, if not all, receptor binding sites with high affinity for the tracer. An autoradiographic [^{35}S]GTPγS-assay was additionally performed to investigate whether putative differences in the numbers of binding sites are also reflected functionally on the level of G-protein activation.

Second, post-mortem tissue allows to further investigate the mechanism by which the *COMT* genotype may relate to the MOP receptor phenotype. As one possible explanation for this interplay between receptor systems, Zubieta et al. (2003) proposed that at least in the striatopallidal system COMT Met$^{108/158}$ related high levels of dopamine may result in the depletion of enkephalin which is one of the endogenous ligands of MOP receptors. As a consequence, MOP receptor expression is upregulated, reciprocally to the level of the endogenous opioidergic ligand enkephalin. Notably, the observation by Zubieta et al. that the release of endogenous opioidergic ligands elicited by painful stimuli is lowest in *COMT* Met$^{108/158}$ homozygous individuals would also be compatible with an assumed role of enkephalin in the regulation of basal MOP binding site expression.

This hypothesis can be tested in post-mortem tissue since the regulation of enkephalin expression can be examined in parallel to changes in MOP binding site expression. Thus, to assess the role of the proposed candidate enkephalin in *COMT* Val$^{108/158}$Met-related MOP receptor function, we determined the regional expression and regulation of preproenkephalin (PPE) mRNA, the transcript for the enkephalin precursor protein.

Materials and methods

Tissue acquisition and slice preparation

Human brain tissues (n = 17) were obtained from Caucasian patients at medicolegal autopsy. None of the patients had a history of neurological or psychiatric disease and all of the females included were in post-menopausal state (Table 1). With a post-mortem interval of mean 17 h, brain tissues were removed for diagnostic reasons. Since tissue availability was limited, we decided to include brain structures whose MOP receptor expression was likely to be influenced by the *COMT* Val$^{108/158}$Met polymorphism—as inferred from the PET study by Zubieta et al. (2003)—like the caudate nucleus, putamen and the nucleus accumbens. In the thalamus, we decided to investigate the mediodorsal nucleus, which is known to express considerable amounts of MOP receptors and which is functionally important for sensory afferent input processing via the medial pain system (Craig, 2003). Moreover, we included BA10 of the frontal cortex and the neocerebellum.

Representative slices from the tissues were prepared and rapidly frozen on dry ice. Sections (20 μm) of brain tissue used in the present study were cut on a cryostat and mounted on poly-L-lysine-coated slides. For in situ hybridization, sections were fixed in 4% paraformaldehyde and stored in ethanol; for both ligand binding techniques unfixed sections were stored at −80°C until experiments were performed. All experiments were performed in duplicate and mean values were used for statistical analyses.

Materials

The radiochemicals [^{3}H][D-Ala^{2},N-Me-Phe^{4},Gly^{5}-ol]enkephalin ([^{3}H]DAMGO, 42.4 Ci/mmol), [^{35}S]guanylyl-5′-(γS)-triphosphate ([^{35}S]GTPγS, 1250 Ci/mmol) and [^{33}P]deoxyadenosin-5′-(αP)-triphosphate ([^{33}P]dATP, 1000 Ci/mmol) were purchased from NEN Perkin-Elmer (Brussels, Belgium). All other chemicals were obtained from Sigma (Taufkirchen, Germany), if not indicated otherwise. Custom-made oligonucleotides were purchased from MWG Biotech (Ebersberg, Germany). The autoradiography films and processing photochemicals were obtained from Kodak (Rochester, NY).

Genotyping

The *COMT* Val$^{108/158}$Met genotypes were determined according to Zubieta et al. (2003; supporting online material) by restriction fragment length analysis.

DNA from cortical tissue samples was isolated by using the Purigene™ isolation kit (Gentra, Minneapolis, MN) according to manufacturer's instruction. A PCR was performed with 30 cycles and by using the forward primer 5′-CTC ATC ACC ATC GAG ATC AA-3′ and the reverse primer 5′-CCA GGT CTG ACA ACG GGT CA-3′, the annealing temperature was 54°C. The resulting 109 bp product was digested with NlaIII (NEB, Beverly, MA) at 37°C for 4 h and visualized by gel electrophoresis (fragment of 86