# Variations in critical morphine biosynthesis genes and their potential to influence human health

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**Abstract**

Endogenous morphine has been detected in human tissues from the vascular, immune and nervous systems. The genes/enzymes (CYP2D6, COMT and PNMT) that are involved in the biosynthesis of morphine have variations that affect their functionality. Some of these variations are the result of single nucleotide polymorphisms of DNA sequences. This review highlights some of the functional differences in the critical enzymes required for the biosynthesis of morphine that may affect human health. These variations have been shown to change the way animals react to stressors, perceive pain and behave. The presence of morphine signaling in almost all organ systems suggests that it is most likely playing a role in maintaining the health and promoting the normal functioning of these physiological systems.

**Abbreviations**

| | |
|---|---|
| MLPC | - multi-lineage progenitor cells |
| DA | - dopamine |
| THP | - tetrahydropapaveroline |
| CYP2D6 | - cytochrome P450 isozyme 2D6 |
| PNMT | - phenylethanolamine N-methyltransferase |
| COMT | - catechol-O-methyltransferase |
| TH | - tyrosine hydroxylase |
| DBH | - dopamine beta-hydroxylase |
| SNP | - single nucleotide polymorphisms |
| NO | - nitric oxide (NO) |
| cNOS | - constitutive nitric oxide synthase |

## INTRODUCTION

The presence of endogenous morphine in animal nervous tissues was suggested as early as the mid 1970's (Gintzler *et al.* 1976; Gintzler *et al.* 1978). Since then, a significant body of empirical evidence has documented the presence of low levels of morphine, its active metabolite morphine-6-glucuronide, and the related morphinan precursor molecules thebaine, salutaridine, norlaudanosoline, reticuline and codeine in animal tissues (Donnerer *et al.* 1986; Lee and Spector 1991; Zhu *et al.* 2003; Kodaira and Spector 1988; Amann and Zenk 1991; Zhu *et al.* 2006b, Zhu *et al.* 2001a, Zhu *et al.* 2002). Although the multi-faceted regulatory functions of endogenous morphine signaling have not been fully characterized, recent work has demonstrated both the presence and functional role of endogenous morphine in immune, neural, endocrine, and cardiovascular systems (Cadet *et al.* 2003; Goumon and Stefano 2000; Zhu *et al.* 2001b).

The primacy of endogenous morphine as a primordial signaling molecule has been compelling suggested by the presence of its cognate mu4 opiate receptor, which is opiate alkaloid selective and opioid peptide insensitive, in human multi-lineage progenitor cells (MLPC) as well as in other diverse cells types (Cadet *et al.* 2007). Complementary microarray analysis of extracted RNA indicated that the proposed morphine biosynthetic enzymes

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are expressed in both undifferentiated and differentiated MLPC (Cadet *et al.* 2007), suggesting that MLPC have the potential for morphine synthesis. The mRNA coding for the enzymes in the morphine biosynthetic pathway, as well as the opiate receptor, exist in progenitor stem cells, suggesting the presence and physiological significance of morphine during early development (Cadet *et al.* 2007).

## BACKGROUND

Historically, morphine has been elevated to a status as an analgesic principle without peer, capable of dramatically reducing human suffering, but bearing an ominously extensive portfolio of debilitating side effects and addictive properties. Recent work has provided evidence that chemically authentic morphine is endogenously synthesized by diverse animal cellular systems from L-tyrosine within a strikingly similar biochemical pathway to that described in opium poppy (Kream and Stefano 2006; Park *et al.* 1999; De-Eknamkul and Zenk 1990; Poeaknapo *et al.* 2004). Furthermore, these reports have empirically demonstrated a functional linkage of *de novo* morphine synthesis to evoked release of the alkaloid upon physiological demand. These accumulated data suggest that low steady-state levels of morphine in many, if not all, mammalian organ systems, indicate dynamic utilization and turnover of releasable cellular pools of morphine, thereby lending support to its essential role as an autocrine/paracrine factor devoted to hierarchal integration of cellular function (Mantione *et al.* 2008; Epple *et al.* 1994).

The biosynthesis of endogenous morphine we now have ascertained in animal cells involves several enzymes that are expressed in tissues of the nervous, endocrine and immune systems (Kream and Stefano, 2006; Zhu *et al.* 2005a, Mantione *et al.* 2008; Poeaknapo *et al.* 2004; Goumon *et al.* 2006; Amann *et al.* 1995). The proposed biosynthetic pathway maintains striking similarities with that recently elucidated in the opium poppy plant (Figure 1)(Kream and Stefano 2006). Initial enzyme steps simulate a classic Pictet-Spengler condensation of dopamine (DA) and 3,4-dihydroxyphenylacetaldehyde to form the benzylisoquinoline compound norlaudanosoline (also called tetrahydropapaveroline, THP) (Figure 1). In the proposed biosynthetic scheme, DA can be derived from ring hydroxylation of tyramine catalyzed by the cytochrome P450 isozyme 2D6 (CYP2D6). The next key enzymatic steps in the synthesis of morphine, methylation of (S)-Norlaudanosoline to (S)-reticuline, have yet to be demonstrated in the laboratory. The 3 sequential methylation steps probably occur via N- and O-methylation events. The proposed enzymes responsible are phenylethanolamine N-methyltransferase (PNMT) and catechol-O-methyltransferase (COMT)(Kream and Stefano 2006). In this regard, N-methyltransferases appear to be important and they represent an ancient class of enzymes required for the methylation of DNA. DNA methyltransferases can control transcriptional events and protect DNA from enzymatic cleavage (Vardimon *et al.* 1983; Vardimon *et al.* 1981; Takahashi *et al.* 2002). PNMT most likely evolved early on in mammals in order to methylate morphine precursors and was later retrofitted to transform norepinephrine to epinephrine (Kream and Stefano, 2006). Invertebrates probably use octopamine in the same way mammals use epinephrine, as it is usually found in the same tissue types and is often associated with norepinephrine (Axelrod and Saavedra 1977; Saavedra and Axelrod 1976).

It has been suggested that morphine synthesis arose during the evolution of animals before the catecholamine synthesis pathway (Stefano and Kream 2007). L-DOPA is the common precursor in the dopamine and morphine biosynthetic pathways. In addition, the terminal catecholamine, epinephrine, has never rigorously been found in plant or invertebrate tissue despite the presence of some reports, which were poorly performed (Stefano and Catapane 1980).

## CRITICAL ENZYMES

With this discussion in mind we speculate that variations of the expression of genes in the morphine biosynthetic pathway may have profound effects on human health since morphine is an endogenous chemical messenger whose presence and absence has not been previously considered in this light. The variations of these genes may have an influence over the way people respond to cognitive and physical stress, especially because DA is a precursor. Polymorphisms in some of the genes in the morphine biosynthesis pathway such as tyrosine hydroxylase (TH), dopa-decarboxylase, dopamine beta-hydroxylase (DBH) and monoamine oxidase A, have not been well studied or linked to function (Haavik *et al.* 2008). PNMT and COMT have been investigated in terms of their effect on mood disorders. For example, the perception of pain is altered by polymorphisms in the COMT gene (Rakvag *et al.* 2005; Zubieta *et al.* 2003) and cardiovascular health can be influenced by changes in the functionality of PNMT (Krizanova *et al.* 2007). In addition, morphine's presence in heart tissue provides further evidence to its critical role in cardiovascular function (Zhu *et al.* 2001b). A recent demonstration by Gavrilovic and co-workers (Gavrilovic *et al.* 2008), indicating that the expression of TH, PNMT and DBH increases in chronically stressed animals, supports the importance of these enzymes in maintaining proper mental health. Interestingly, endogenous morphine release and levels are enhanced following surgical, environmental, and psychological stressors (Brix-Christensen *et al.* 1997; Mantione *et al.* 2003; Cadet *et al.* 2002; Narita *et al.* 2006), suggesting their presence is required to mediate, at least in part, some aspect of this response.

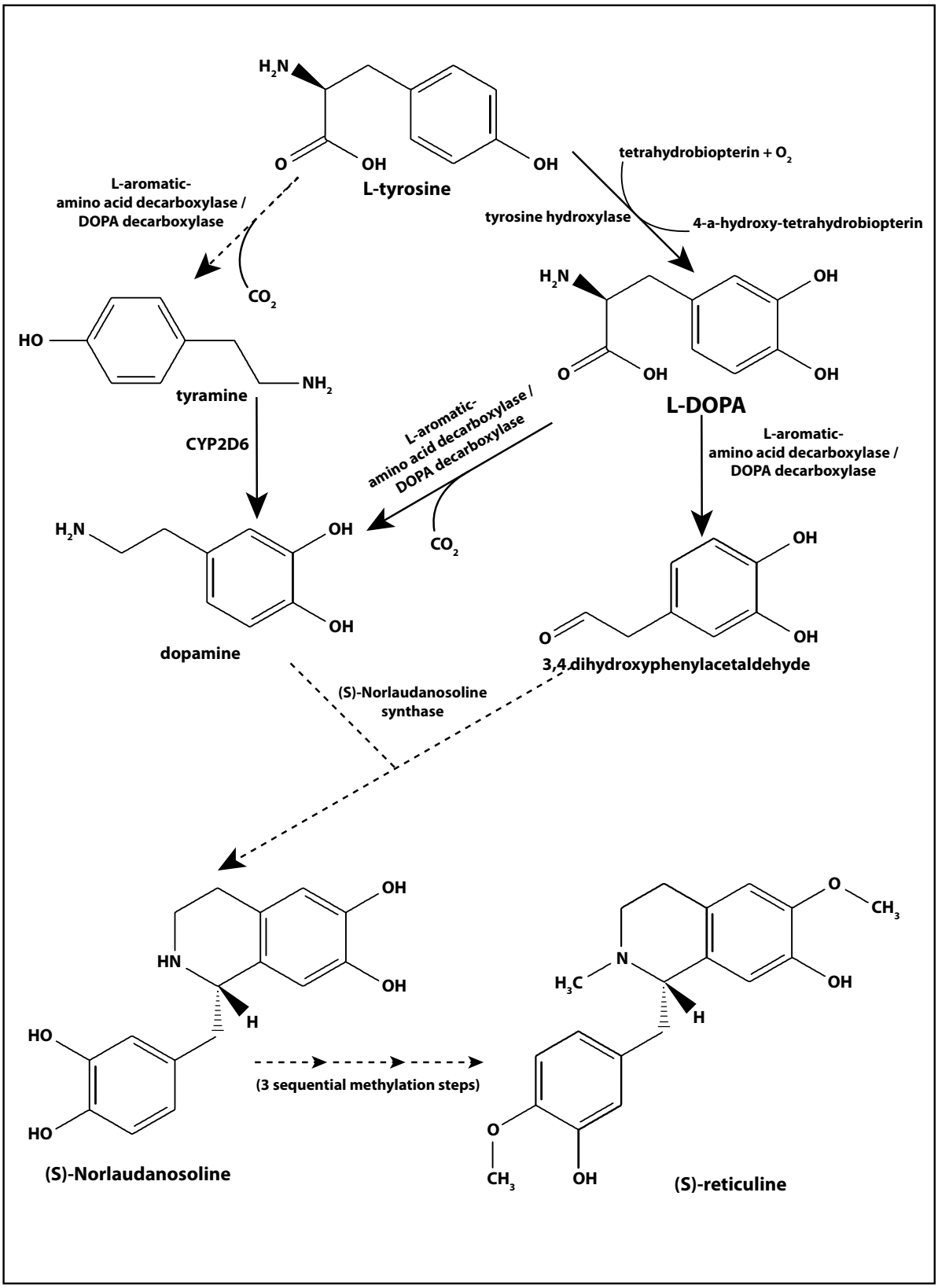


**Fig. 1.** The initial steps in the biosynthesis of morphine leading to the formation of reticuline (reproduced from Kream and Stefano 2006).

Mood disorders, such as aggression, depression, schizophrenia, and attention deficit/hyperactivity disorder could be linked to mutations in the genes of the morphine biosynthetic pathway via their role in DA metabolism or via a direct role in the biosynthesis of morphine post-DA (Yamano *et al.* 2008; Lachman 2008; Cohen and Pickar 1981; De *et al.* 2006; Schmauss and Emrich 1985; Halleland *et al.* 2009; Kopeckova *et al.* 2008). In a recent investigation of COMT haplotypes in a population of Chinese schizophrenics it was concluded that violent behavior could be predicted by genotype (Gu *et al.* 2009). The propensity of individuals to become addicted to opiates as well as to alcohol, cocaine, amphetamines or nicotine most likely depends on their genotype (Oosterhuis *et al.* 2008). Behavioral characteristics have recently been investigated with regard to PNMT single nucleotide polymorphisms (SNP). In a Japanese female sub-population, Yamano and co-workers (Yamano *et al.* 2008) found that reward dependence personality trait may be associated with a PNMT SNP.

Addiction to opiates may be caused by the loss of normal function of the enzymes in the morphine biosynthetic pathway, i.e., a morphine insufficiency syndrome (Stefano and Scharrer 1994). Recent work has indicated that nicotine, cocaine and alcohol, in part, appear to exert their pleasure-providing action via a common process, which involves the stimulation of morphine release or production from its cellular sites of synthesis (Zhu *et al.* 2006a). It has been shown that morphine and its downstream signaling molecule, nitric oxide (NO), can influence the transcription of COMT as well as CYP2D6 (Mantione *et al.* 2008), providing for negative feedback modulation, which may be abnormally operating if these chemical messengers arise from an exogenous source. Tolerance to drugs of abuse (including opiates) that affect endogenous morphine signaling should be viewed as a protective mechanism. The attenuation of the action of morphine's signal ensures that the normal inhibitory effects in the nervous, vascular and immune systems does not compromise these systems for an extended period of time (Stefano *et al.* 2009a;b).

*CYP2D6*

Of all the enzymes involved in the biosynthesis of morphine, CYP2D6 and its variations are very important and, because of the diverse catalytic functions it may serve, the most difficult to ground in an absolute morphinergic process. However, Zhu *et al.* (2005b) demonstrated that exposing *Mytilus edulis,* a marine bivalve mollusk, pedal ganglia to the putative morphine precursors, tyrosine and tyramine resulted in significant increases in ganglionic morphine levels, which are time and concentration dependent. The authors demonstrated that CYP2D6 and TH are involved in this process. CYP2D6 presence and its role in this process is further supported by the finding of many cytochrome P450 isoforms in *M. edulis* tissues (Snyder 2000). It catalyzes the conversion of reticuline to salutaridine (Amann *et al.* 1995). In addition, CYP450 isoforms have been found that convert codeine to morphine (Pai *et al.* 2004) and tyramine to dopamine (Hiroi *et al.* 1998). Quinidine, a specific inhibitor of CYP450, inhibited the pathway and was shown to exert this action in the conversion of tyramine to dopamine, reticuline, THP, DA and codeine metabolism to morphine (Zhu *et al.* 2005b, Zhu *et al.* 2005a). These findings also support the presence of this enzyme in this invertebrate ganglion since the isolated mRNA fragment and resulting RT-PCR product, exhibits 94% sequence identity with its human counterpart (Pai *et al.* 2004). These results place CYP2D6 in a pivotal position in the biosynthesis of morphine in animals (Figure 1).

As demonstrated, neither alpha-methyl-para-tyrosine nor quinidine when administered alone dropped endogenous morphine levels below that of controls (Zhu *et al.* 2005b, Zhu *et al.* 2005a). Co-administration did however cause this to occur. The enzyme inhibitor data suggest that if one pathway is blocked, the overall pathway continued because the other pathway to dopamine compensated. In this regard, it appears that morphine biosynthesis is such a vital process that it can occur from two starting points, ensuring its production.

Considering the multiple roles that CYP2D6 can play in the synthesis of morphine, the enzyme's activity may have control over the rate of production of morphine. Candiotti and co-workers (Candiotti *et al.* 2009) investigated groups of patients by comparing CYP2D6 metabolic rates. Those patients with the genotype for a rapidly acting CYP2D6 required less morphine for post operative pain than individuals with the slower metabolizing forms of the enzyme (Candiotti *et al.* 2009). The researchers speculated that the rapid metabolizers probably had a greater production of morphine than the slower metabolizers. In other cases with patients possessing rapidly acting CYP2D6, adverse reaction to codeine have been reported (Gasche *et al.* 2004). Codeine is easily demethylated to morphine by CYP2D6 (Pai *et al.* 2004; Zhu *et al.* 2005a). Codeine's analgesic effect is dependent on its conversion to morphine by CYP2D6 (Zwisler *et al.* 2009). In individuals with ultra-rapid CYP2D6, codeine used for treatment of cough can cause life threatening respiratory depression (Gasche *et al.* 2004). Conversely, patients who possess the genotype for a poor metabolizing CYP2D6 do not efficiently convert codeine to morphine and trying to manage pain in these patients is complicated (Foster *et al.* 2007; Oertel and Lotsch 2008; Zwisler *et al.* 2009).

*PNMT*

The enzyme PNMT is most likely responsible for some of the methylation reactions needed for morphine biosynthesis (Kream and Stefano, 2006). PNMT's most well studied function is the production of epinephrine. PNMT is present in high concentrations in the adrenal medulla,

but it can also be found in nervous and cardiac tissues (Ziegler MG *et al.* 2002). Tissues of the adrenal medulla as well as sympathetic nerves are both derived during development from neural crest cells (Souto and Mariani 1996). PNMT mRNA expression can be up regulated by stress hormones (Kubovcakova *et al.* 2006). Similarly, morphine levels are increased by exposure to stress hormones (Cadet *et al.* 2003). The presence of PNMT in *M. edulis* neural tissue and the lack of epinephrine (Stefano and Catapane 1980) in these animals also suggest a role for PNMT in morphine synthesis. The presence of morphine in an adrenal chromaffin cell line (Goumon *et al.* 2000) and in rat adrenal glands (Goumon and Stefano 2000) as well as the secretion of morphine-6-glucuronide by adrenal chromaffin cells (Goumon *et al.* 2006) suggests the involvement of PNMT, and other enzymes found in the adrenal glands, in morphinergic processes.

Morphine and epinephrine levels can be increased by stress hormones (Cadet *et al.* 2003; Kubovcakova *et al.* 2006; Nakamura *et al.* 2005), placing these chemical messengers in an important survival pathway. In rats without pituitary glands, the glucocorticoid, dexamethasone, stimulated PNMT mRNA production (Evinger *et al.* 1992). Conversely, decreased corticosteroids have been linked to decreased PNMT activity (Wong *et al.* 1995). In adrenal glands, glucocorticoids are needed for PNMT expression (Ziegler *et al.* 2002). Changes in the hypothalamic-pituitary-adrenal axis are typical in depressed patients. Specifically, hyper-activation of the hypothalamic-pituitary-adrenal axis due to loss of functionality of the glucocorticoid receptor can lead to major depression (Nikisch 2009). Corticotropin releasing hormone knockout mice do not up regulate their PNMT mRNA in response to stress (Kvetnansky *et al.* 2008). The presence of normal glucocorticoid signaling may be necessary for a functional morphine biosynthetic system. The suggestion by Nikisch (Nikisch 2009) that restoring and maintaining normal glucocorticoid receptor signaling in treating depression supports the importance of the morphine biosynthetic enzyme PNMT for mental health. It also suggests that preserving proper morphine signaling is vital to treating mood disorders.

If PNMT is serving to synthesize morphine as well as epinephrine, there might be multiple forms of this enzyme to perform these actions. Different forms of PNMT mRNA exist in adrenal glands and in cardiac tissue (Ziegler *et al.* 2002). The heart contains two forms, one containing introns and the other is an intronless message. The adrenal gland only contains the intronless form (Ziegler *et al.* 2002; Unsworth *et al.* 1999). The intronless form's expression is increased by dexamethasone and the intron containing form is decreased (Unsworth *et al.* 1999). In the heart, the intronless message can increase blood pressure, glucose and lymphocyte cytokine production (Unsworth *et al.* 1999). The PNMT responsible for morphine synthesis has yet to be characterized.

Alcohol intake affects the cardiovascular system differently based on polymorphisms of PNMT and COMT (Nishimura *et al.* 2008). TH, DBH and PNMT were studied with respect to blood pressure effects in rats. In rats with hypertension, PNMT expression was found to be higher during the light cycle (Zeman *et al.* 2008). Given that morphine is present in the heart and its receptor is also found in the heart and vasculature, morphine production and signaling is likely influencing this system (Zhu *et al.* 2001b).

*COMT*

COMT has been implicated in morphine biosynthesis in both invertebrates and vertebrates (Kream and Stefano 2006), making it highly significant, especially since it exhibits polymorphisms. The most studied COMT polymorphism is termed val/met 158. This polymorphism has a methionine substituted for a valine at amino acid 158 (Syvanen *et al.* 1997). Future ongoing studies are attempting to establish a link between this polymorphism and behavior (Lachman 2008). The effect of this polymorphism is a lowering of the activity of COMT and thus a slower metabolism of dopamine (Syvanen *et al.* 1997; Kunugi *et al.* 1997). Nackley and co-workers (Nackley *et al.* 2006) studied the structure of COMT mRNA and linked its stable structure to low COMT protein levels. COMT val/met 158 homozygotes for the met allele have a higher sensory and affective rating for pain due to diminished μ-opioid receptor responses (Zubieta *et al.* 2003). Mutations in the μ-opioid receptor, as well as genes, are thought to be involved with morphine synthesis. Guanosine 5'-triphosphate cyclohydrolase, COMT, and CYP2D6, have all been reported to affect pain perception as reviewed by Oertal *et al.* (2008).

Individuals possessing the COMT val/met 158 phenotype have a lower pain threshold, but these patients also require less exogenous morphine for pain relief, creating a novel paradox (Rakvag *et al.* 2005). These patients have a slower clearance time for dopamine and therefore may have more dopamine in the synapses available for morphine biosynthesis. Perhaps high dopamine could contribute to a lower pain threshold due to increased conversion to endogenous morphine and subsequent down regulation of the μ-opioid receptor (i.e., tolerance). An alternate explanation as to why these individuals needed less exogenous morphine could be that the low COMT activity might lower the production of endogenous morphine and therefore up regulate the morphine receptor, however, this does not explain why less morphine is required for analgesia. Additionally, lower morphine levels may initiate a compensatory response in opioid peptide levels, adapting to the morphine level alteration.

Furthermore, higher than normal pain sensitivity might be caused by increased beta 2 and beta 3 adrenergic receptor activation (Diatchenko *et al.* 2005; Nackley *et al.* 2007). These receptors could be hyper-activated due to the elevated catecholamine levels in individuals

with a defective COMT gene. In a report by Nackley and co-workers (Nackley *et al.* 2007), selective adrenergic receptor antagonists blocked the heightened pain responses in rats. Another study in patients with Crohn's disease found that this group had a greater need for opioids for pain relief (Huehne *et al.* 2009). The greater need was not found to be linked with any SNP for low COMT activity or μ-opioid receptor variants (Huehne *et al.* 2009). A possible explanation for diminished COMT activity in these cases may be provided by Tchivileva and co-workers (Tchivileva *et al.* 2009). Nuclear factor kappa beta activation due to the presence of tumor necrosis factor alpha was found to down regulate both mRNA and protein levels of COMT (Tchivileva *et al.* 2009). Lower COMT activity might diminish endogenous morphine production and lead to greater need for exogenous morphine for the treatment of Crohn's disease.

If decreased COMT activity is associated with low morphine production, then it may impact morphine signaling pathways. It has been established that morphine stimulates constitutive nitric oxide synthase (cNOS) via the mu opiate receptor (Stefano *et al.* 1995; Zhu *et al.* 2004; Mantione *et al.* 2002). Thus, in a system lacking morphine, NO production may be diminished. NO is important in the regulation of blood pressure via its actions on blood vessels (Ignarro *et al.* 1981; Omawari *et al.* 1996; Pechanova *et al.* 2009; Moncada, 1994). The diminished NO levels due to a lack of morphine signaling could be a factor in the development of hypertension. In a study of middle aged men in Sweden, subjects with the COMT val/met 158 polymorphism had higher blood pressure than the those with the normal COMT genotype (Annerbrink *et al.* 2008). Recently, COMT deficiency has been implicated in the development of pre-eclampsia (Kanasaki *et al.* 2008). Blood pressure can be reduced in subjects given morphine for treatment of pre-eclampsia (Costas *et al.* 1986). Future research is needed to establish a link between morphine signaling and the development of pre-eclampsia.

## CONCLUSION

The biomedical significance of morphine is only now beginning to be understood. The presence of morphinergic signaling in numerous organ systems provides compelling evidence for the profound impact that morphine may have on human health, transcending analgesic processes. The pivotal role of L-DOPA/DA in endogenous morphine biosynthesis and its coupling to cNOS derived NO release via novel mu-opiate receptor subtypes establishes opiate alkaloid signaling as important. Therefore, variations in the genotypes and/or expression of enzymes in the morphine biosynthetic pathway offer critical relevance in considering pathological processes involved with mental and physical health.

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