CYTOCHROME P450 2E1 – RELEVANCE FOR CENTRAL DOPAMINE NEUROTRANSMISSION AND PARKINSON'S DISEASE

Haydeh Niazi Shahabi
2008

UNIVERSITY OF GOTHENBURG

Department of Pharmacology
Institute of Neuroscience and Physiology
The Sahlgrenska Academy at University of Gothenburg
Sweden

Printed by Chalmers Reproservice, Göteborg, Sweden

ISBN 978-91-628-7542-8

Abstract

CYTOCHROME P450 2E1 – RELEVANCE FOR CENTRAL DOPAMINE NEUROTRANSMISSION AND PARKINSON'S DISEASE

Haydeh Niazi Shahabi

Department of Pharmacology, Institute of Neuroscience and Physiology,
The Sahlgrenska Academy, University of Gothenburg, Box 431, SE-405 30, Göteborg, Sweden

Introduction: The enzyme cytochrome P450 2E1 (CYP2E1) has been found in dopamine (DA) containing brain regions that are of relevance for Parkinson's disease (PD), and is known to generate reactive oxygen species (ROS), toxic molecules that have been implicated in the degeneration of DA neurones. In addition, previous investigations have indicated that inhibition of CYP2E1 increases extracellular DA in the substantia nigra, a nucleus which degenerates in PD. It is therefore of interest to elucidate a possible involvement of CYP2E1 in DA metabolism/neurotransmission and participation in producing ROS. Furthermore, CYP2E1 gene polymorphisms have been reported, variations which could influence susceptibility to PD. Thus, an inspection of polymorphic variants in a population of PD patients as compared to controls was conducted. ***Methods and observations:*** By injection of the radioactive DA precursor L-DOPA to rats in vivo, major catecholamines and their metabolites could be separated and quantitatively examined for radioactivity utilizing a liquid chromatography system. Inhibition of CYP2E1 induced significant changes in the radioactivity pattern. Moreover, the increase in extracellular DA in the substantia nigra, measured by in vivo microdialysis in rats, induced by CYP2E1 inhibition was unaltered following pharmacological inhibition of DA neurone firing and the DA transporter. Tetrodotoxin or reserpine treatment conversely abolished this effect. In addition, an increase in ROS production in the substantia nigra was observed during the presence of an exogenous CYP2E1 substrate (isoflurane). Investigation of polymorphic forms of CYP2E1 was carried out via a tag-single nucleotide polymorphism approach, obtaining Haplotype block data. An association between a C/G polymorphism at intron 7 of this gene and PD was found. Furthermore, extraction of genomic CYP2E1 RNA from putamen/caudate nucleus of five individuals revealed two alternatively spliced variants. ***Conclusions:*** The results support the notion that CYP2E1 is located near or in the same compartment as stored DA in the substantia nigra, possibly modulating DA neurotransmission and generation of ROS. Furthermore, inspection of polymorphic forms of CYP2E1 revealed a possible association of this enzyme with PD. Finally, we show that both intra- and inter-nuclei alternatively spliced variants of CYP2E1 exist in brain parts that are of relevance for PD pathophysiology.

Keywords: cytochrome P450 2E1, dopamine, substantia nigra, polymorphism, alternative splicing.

ISBN 978-91-628-7542-8

This thesis is based on the following papers:

Paper I: **Niazi Shahabi H.**, Bergquist F. and Nissbrandt H. An investigation of dopaminergic metabolites in the striatum and in the substantia nigra in vivo utilising radiolabelled L-DOPA and high performance liquid chromatography: A new approach in the search for transmitter metabolites. *Neuroscience 120: 425-433, 2003*

Paper II: **Niazi Shahabi H.**, Andersson D. R. and Nissbrandt H. Cytochrome P450 2E1 in the substantia nigra: Relevance for dopaminergic neurotransmission and free radical production. *Synapse 62:379-388, 2008*

Paper III: **Niazi Shahabi H.**, Westberg L., Melke J., Håkansson A., Carmine Belin A., Sydow O., Olson L., Holmberg B., and Nissbrandt H. Cytochrome P450 2E1 gene polymorphisms/haplotypes and Parkinson's disease in a Swedish population. *Submitted, 2008*

Paper IV: **Niazi Shahabi H.**, Melke J., Nordborg C., Kjellström C. and Nissbrandt H. Expression of alternatively spliced cytochrome P450 2E1 in the putamen and the caudate nucleus of human brain. *Manuscript, 2008*

List of abbreviations

AADC	aromatic L-amino acid decarboxylase
α-MD	α-methylDOPA
cDNA	complementary deoxyribonucleic acid
COMT	catechol-O-methyltransferase
CYP 2E1	cytochrome P450 2E1
CYP450	cytochrome P450
DA	dopamine
DNA	deoxyribonucleic acid
DOMA	3, 4-dihydroxymandelic acid
DOPAC	3, 4-dihydroxyphenylacetic acid
DOPEG	3, 4-dihydroxyphenylglycol
DPM	disintegration per minute
ER	endoplasmatic reticulum
GABA	γ-aminobutyric acid
5-HIAA	5-hydroxyindoleacetic acid
HPLC	high performance liquid chromatography
5-HT	5-hydroxytryptamine = serotonin
HVA	homovanillic acid
L-DOPA	L-3, 4-dihydroxyphenylalanine
MAO	monoamine oxidase
MMFO	microsomal mixed function oxidase
MOPEG	3-methoxy-4-hydroxy-phenylglycol
MOPET	3-methoxy-4-hydroxyphenylethanol
mRNA	messenger ribonucleic acid
3-MT	3-methoxytyramine
NE	norepinephrine
NM	normetanephrine
3-OMD	3-O-methyl-DOPA
PCR	polymerase chain reaction
PEITC	phenylethyl isothiocyanate
PD	Parkinson's disease
RNA	ribonucleic acid
ROS	reactive oxygen species
SN	substantia nigra
SNP	single nucleotide polymorphism
TH	tyrosine hydroxylase
tSNP	tag-single nucleotide polymorphism
VMA	3-methoxy-4-hydroxy-mandelic acid
VMAT	vesicular monoamine transporter

TABLE OF CONTENTS

PREFACE

Simorgh (سیمرغ)

It is told in Persian mythology that a gigantic yet graceful female bird-like creature called Simorgh, having fire glowing wings of thirty colours and being the size of thirty birds, had seen the rise and fall of the world three times, gaining more and more wisdom and compassion. It sat on the mighty and only tree in the middle of the sea of life, loving water, earth and sky. This mighty tree was made up of all trees and plants, bearing the cure to all that caused illness. When Simorgh finally took to the sky, being the powerful bird that she was, the tree shook impetuously and was completely scattered around the world, falling to root … and grew. Hence, cure for all diseases exist in the plants, waiting to be discovered.

We have to believe that there are answers to the questions of science and the hope to heal will never fade. Weather it is because of stories of Asclepius, the Greek God of medicine, healing through his dreams or Simorgh spreading seeds of cure, man has always nurtured the faith in his own knowledge to solve problems. That is the driving force of all who seek answers. The present thesis is a tiny contribution to this knowledge and gratitude towards those who encouraged me to nurture this faith in my thoughts throughout the path of life.

INTRODUCTION

GENERAL BACKGROUND

Parkinsons's disease (PD) is the second most common neurodegenerative disease. The typical motor deficit symptoms are mainly due to the degeneration of dopamine (DA) containing neurones projecting from the substantia nigra to the striatum. The cause of the degeneration of DA-containing neurones in PD are, however, largely unknown. It is generally believed that in most cases environmental factors interact with genetic constitution. Gene mutations have been shown to cause familial PD, and some susceptibility genes have been discovered. Several environmental factors have been suggested to be of importance, however, not yet has any single or cluster of factors unequivocally been shown to be associated with sporadic PD. There is a vast amount of experimental studies giving support for various pathophysiological mechanisms involved in the neurodegenerative process. Among several mechanisms are increased generation of reactive oxygen species, decreased defence against reactive oxygen species, inhibition of complex I of the respiratory electron transport chain by toxins, defects in iron metabolism, increase in intracellular calcium due to excessive glutamate, and apoptosis. Presently, there is no curative or cytoprotective treatment available but several drugs with symptomatic effect can be used. In spite of such treatments, after several years of disease the patients often have difficulties with activities of daily living.

The cytochrome P450 family of enzymes are mainly found in the liver where they metabolise a wide variety of endogenous substances and detoxify exogenous toxins. Some enzymes in this family, e.g. cytochrome P450 2E1 (CYP2E1), are also present in some extra hepatic tissue such as kidney, lung and brain. This enzyme has been detected in different brain regions including the substantia nigra. Previous findings indicate that this enzyme may influence dopaminergic neurotransmission in the brain and generate toxic reactive oxygen species, which have been implicated to contribute to the pathophysiology of PD. One aim of this thesis was to investigate the influence of CYP2E1 activity on DA metabolism and release in rat brain. Differences in the control of CYP2E1 activity as a consequence of genetic variation can theoretically alter its production of reactive oxygen species, or change the metabolic capacity and hence the defending ability of the tissue against possible harmful substances. Therefore, it is of interest to investigate possible associations between CYP2E1 gene polymorphisms and PD. Thus, an additional aim was to inspect polymorphic forms of the CYP2E1 gene in a population of patients with PD in comparison to healthy controls and also to verify the existence of diverse forms of the enzyme in relevant brain nuclei in humans.

DOPAMINE AND THE BASAL GANGLIA

The breakthrough and significance of dopamine

James Parkinson (1785-1824) published "*An assay on the shaking palsy"* in 1817, describing in detail and with precision, what was later to be called "Parkinson's" disease. Not only did he portray the apparent symptoms of this disease, but he also explained the difficulty of living with these symptoms for the observed patients. He wrote about one patient:
"… and at the last, constant sleepiness, with slight delirium, and other marks of extreme exhaustion, announce the wished-for release."

PD has since then been characterized and investigated by many who share the common intension of finding the cause and hence best suitable therapy for the affected patients. With the growing interest for the substance referred to as 3-hydroxytyramine (Carlsson, 2002; Hornykiewicz, 1986), began the history of modern experimental research on PD. This substance was later to be called DA and is yet among the most studied neurotransmitters in the brain.

The story of this neurotransmitter started with the synthesis of the substance in 1910 by Barger and Ewens. Funk synthesized D,L-3,4-dihydroxyphenylalanine (D-,L-DOPA), the racemic form of the DA precursor in 1911, and later Guggenheim (1913) isolated L-DOPA from the seedlings of *Vicia faba*. He also suggested that this amino acid was a precursor of epinephrine which some years later lead to the proposal of a pathway for catecholamine synthesis by Holtz (1939) and by Blaschko (1939). Previously, in 1938 Holtz discovered DOPA decarboxylase, the enzyme converting DOPA to DA. During this time, DA was considered merely as an intermediate in the synthesis of norepinephrine (NE). Although Blaschko considered the possibility of a physiological function for DA, it was not until Carlsson (1958) discovered the occurrence of DA in the brain that a new era in research regarding PD began. Carlsson developed a more sensitive fluorimetric method for measuring DA and showed that the amount of this substance in the brain of rabbits is much higher than would be expected of a precursor to NE. He also observed that rabbits treated with the already known drug reserpine (the active substance of the Indian plant *Rauwolfia serpentina*) could after administration of L-DOPA regain motor activity and performance together with a rise in DA concentration, while NE levels were much less influenced by L-DOPA treatment. These findings led Carlsson to propose that DA is not simply a precursor of NE but has a physiological role of its own. When Bertler and Rosengren (1959) showed that the regional distribution of DA in the brain (especially in the basal ganglia) of different species did not necessarily coincide with high amounts of NE, they suggested the involvement of DA as a neurotransmitter in the control of movement. It was soon thereafter, when Ehringer and Hornykiewicz (1960)

compared human brain samples of Parkinson patients with normal brain tissue using the chemical assay earlier developed by Carlsson, that evidence for a connection between DA deficiency and PD was obtained. A year later Barbeau, Murphy and Sourkes (1961) showed that the urine of Parkinson patients contained only low concentration of DA and thereafter, several groups of scientists began treating patients with L-DOPA (Barbeau et al., 1962; Birkmayer and Hornykiewicz, 1962; Cotzias et al., 1967). Administration of this amino acid is yet the most effective therapy in PD.

The basal ganglia

The basal ganglia are a group of interrelated nuclei consisting of the *caudate nucleus* and the *putamen* (which together build up the striatum), globus pallidus (containing internal and external segments), subthalamic nucleus and substantia nigra. The substantia nigra is further divided into two zones, the lighter zone of *pars reticulate* and a darker pigmented zone called *pars compacta*. There are some anatomical differences between the basal ganglia of primates and the rat, such as a less obvious distinction between the two structures of the striatum and the two segments of the globus pallidus in the rat. In general, the striatum receives afferent input mainly from the cortex, while the globus pallidus and the substantia nigra pars reticulata function as major output nuclei of the basal ganglia and send projections to the thalamus and superior colliculus (Heimer and Alheid, 1985; Mink, 1999). Neurochemically distinct and lightly stained patches called striosomes are seen in the striatum, which receive limbic projections. From these striosomes, project neurones to the dopaminergic neurones of the substantia nigra pars compacta. A darker stained part of striatal tissue called the matrix receives input from the cortex and sends projections into the substantia nigra pars reticiulata (Heimer and Alheid, 1985). The basal ganglia nuclei mainly manage the control of motor activity and eye movement, but may also be important for cognitive functions. DA is the major monoamine in the basal ganglia with the highest concentration in the striatum. A simplified illustration of the input, output and interconnections of the basal ganglia are shown in Fig.1.

According to this model there are two major pathways in the basal ganglia. One pathway starts with the inhibitory output neurones (γ-aminobutyric acid) from the striatum through the internal segment of the globus pallidus and substantia nigra pars reticulata, to the thalamus. High activity in the striatal output neurones through the two former nuclei results in a decrease of the inhibitory input of the basal ganglia to the thalamus and is referred to as the direct route. The other pathway called the indirect route applies to the path from the striatum towards the globus pallidus external segment and thereon to the subthalamic nucleus. This latter nucleus sends excitatory (glutamic acid) projections into globus pallidus internal segment and substantia nigra pars reticulata, to further join and

follow the direct route into the thalamus. The effect of stimulation of the indirect route is an increase of the inhibitory input of the basal ganglia on the thalamus.

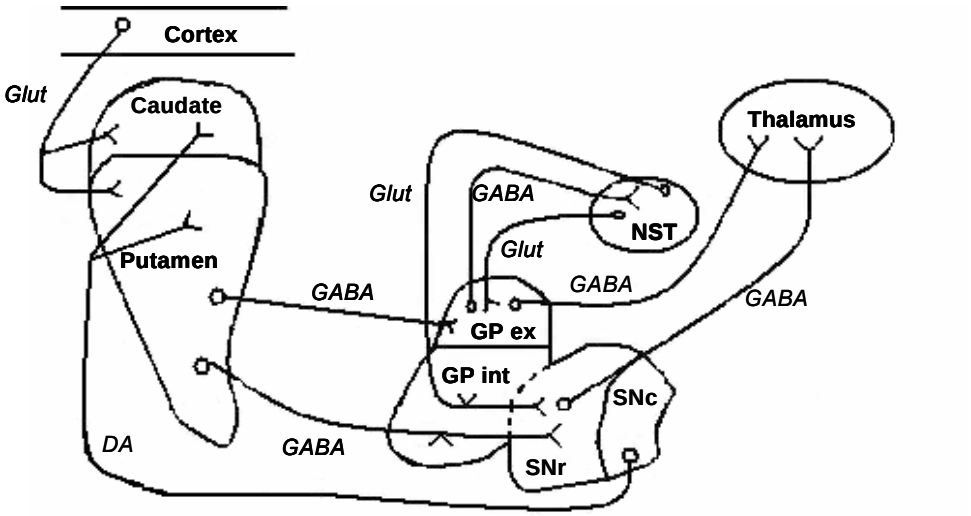

Figure 1. *A simplified schematic illustration of the basal ganglia and its connections. Abbreviations: DA, Dopamine; GABA, γ-aminobutyric acid; Glut, Glutamic acid; GP int, Globus pallidus internal segment; GP ex, Globus pallidus external segment; NST, Subthalamic nucleus; SNc, Substantia nigra pars compacta; SNr, Substantia nigra pars reticulate.*

This model has been used to illustrate the main functional organisation of the basal ganglia. Release of DA from the terminals of the nigrostriatal pathway stimulates mainly two types of DA receptors. These are the excitatory D_1 receptors (expressed primarily at the direct route of the striatal neurones) and the inhibitory D2 receptors (expressed mainly at the indirect route of the striatal neurones). These receptors stimulate/inhibit *inter alia* the synthesis of adenylyl cyclase (and as a result cAMP), respectively, having a complementary and additive stimulatory effect on the motor activity. The degeneration of dopaminergic nigro-striatal neurones in PD results in a decrease of DA release in the striatum. This in turn decreases the activity of the direct pathway due to lack of D_1 receptor stimulation while lesser activity of the D_2 receptors leads to an increase in the outflow from the indirect route (Standaert and Young, 1996). The consequence of this DA deficiency in PD is the appearance of hypo- and brady-kinesia.

Synthesis and metabolism of dopamine

The enzyme tyrosine hydroxylase (TH) catalyses the first step in the synthesis of DA, transforming the amino acid tyrosine to L-DOPA. TH has been found in the cytoplasm and in association with the endoplasmatic reticulum in the substantia nigra (Hattori et al., 1979; Pickel et al., 1977), whereas in the axon terminals in the striatum it is localized in the cytoplasm close to the vesicles (Pickel et al., 1981). The action of TH, which is the rate limiting step in the synthesis of catecholamines, is regulated by the firing rate of the dopaminergic neurones, DA autoreceptor occupancy and by end-product inhibition, in this case DA. It is normally saturated with its substrate and approximately only 2% of the available tyrosine is utilised for catecholamine biosynthesis (Cooper et al., 1996). The synthesised L-DOPA is then converted to DA by aromatic L-amino acid decarboxylase (AADC). This enzyme is not saturated with substrate (Bowsher and Henry, 1985) and is expressed in dopaminergic, noradrenergic and serotonergic neurones, and has also been detected in rat glia cells (Li et al., 1992; Nakamura et al., 2000). The function of the enzyme in the latter cells is however, not clear.

DA metabolism occurs mainly through oxidative deamination by monoamine oxidase (MAO) and O-methylation by catechol-O-methyltransferase (COMT). They convert DA to 3, 4-dihydroxyphenylacetic acid (DOPAC) (Rosengren, 1960) and 3-methoxytyramine (3-MT) (Carlsson and Waldeck, 1964), respectively, which in turn are further metabolised to homovanillic acid (HVA) as their end product (Rutledge and Johanson, 1967). DA is also a precursor to NE. A more detailed description of the metabolism of DA and NE is schematically illustrated in Fig. 2.

DA neurones in the substantia nigra of rat brain contain higher concentration of MAO-A than MAO-B mRNA (Jahng et al., 1997). The former type is apparent both inside and outside synaptosomes in the striatum of rat, contributing to deamination of NE and DA (Fagervall and Ross, 1986). Differences between human and rat brain are seen, such as a higher extra-synaptosomal activity of MAO-B in humans (Stenstrom et al., 1987), as compared to MAO-A activity. O-Methylation of DA takes place outside catecholamine neurones and the soluble form of COMT (S-COMT) is the dominant enzyme in both rat and human brain (Guldberg and Marsden, 1975).

The possibility of alternative pathways leading to other DA metabolites have also been considered and investigated; e.g. DA glucuronid (Wang et al., 1983) and sulfoconjugated DA (Buu et al., 1981) have been detected and are probably metabolites of minor quantitative importance.

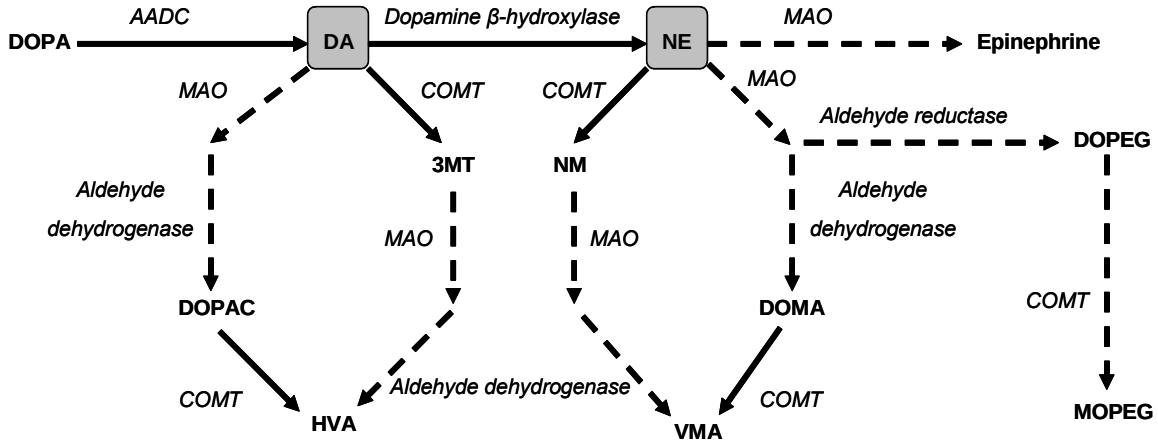

Figure 2. *A Schematic presentation of the catabolism of dopamine and norepinephrine. Abbreviations: DA, dopamine; DOPA, dihydroxyphenylalanine; NE, norepinephrine; DOMA, 3,4-dihydroxymandelic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPEG, 3,4-dihydroxyphenylglycol; MOPEG, 3-methoxy-4-hydroxy-phenylglycol; 3MT, 3-methoxytyramine; NM, normetanephrine; HVA, homovanillic acid; VMA, 3-methoxy-4-hydroxy-mandelic acid; COMT, catechol O-methyl transferase, MAO, monoamine oxidase. Dashed lines indicate the presence of more than one step in the current path.*

Storage and release of dopamine

Histological studies of false transmitter (5-hydroxydopamine) storage in the substantia nigra indicated that the main storage site for DA in dendrites is not in vesicular structures, which is the case in terminal parts such as the striatum, but in the smooth endoplasmatic reticulum (SER), also apparent as a storage site for DA in cell bodies in combination with vesicular storage (Groves and Linder, 1983; Hattori et al., 1979; Mercer et al., 1979; Wilson et al., 1977). An immunohistochemical study of the localization of the vesicular monoamine transporter-2 (VMAT2), the carrier of DA into the vesicles in axon terminals, supported these findings (Nirenberg et al., 1996). As in dopaminergic terminal regions, inhibition of the DA re-uptake mechanism (DAT = dopamine transporter) increases the extracellular concentrations of DA in the substantia nigra, stressing the importance of DAT also in this brain part (Engberg et al., 1997; Sarre et al., 2004). Administration of γ-butyrolactone (GBL), which decreases or abolishes the firing of dopaminergic neurones, has a modest inhibitory effect on DA release in the substantia nigra (Hefti et al., 1976; Pericic and Walters, 1976; Santiago and Westerink, 1991, 1992), whereas in the striatum, a more apparent decrease is seen following systemic administration of this drug (Imperato and Di Chiara, 1984; Santiago and Westerink, 1991, 1992). As in the striatum, nigral dopamine release is dependent on

voltage-dependent sodium and calcium channels (Bergquist et al., 1998; Santiago and Westerink, 1992). The modulatory effect of DA autoreceptors on DA release has been found to be less pronounced in the substantia nigra as compared to the striatum (Hoffman and Gerhardt, 1999; Nissbrandt et al., 1989; Pucak and Grace, 1994; Santiago and Westerink, 1991).

PARKINSON'S DISEASE

Signs and symptoms

The prevalence of PD is 1.6-1.8% over the age of 65, rising to 2.6% for those older than 85 years (de Rijk et al., 2000). The clinical diagnosis is founded on four main symptoms which are hypo- bradykinesia (impaired ability to start or continue movement), loss of postural stability, muscular rigidity and resting tremors. The latter symptom can ease by movement. With progression of the disease the patient shows a flexed posture, experiences the freezing phenomenon (the legs are glued to the ground) and eye tracking movement may be lost. These last symptoms are often referred to as non-DA related. A noticeable clinical response to L-DOPA therapy often confirms the diagnosis.

Pathological findings

It was already in 1895 that Brissaud suggested an association of substantia nigra with PD. Later Lewy (1914) detected cytoplasmic inclusions, now known as Lewy bodies, in the brain of patients with this disorder. The neuropathology of PD was studied in more detail by Trétiakoff in 1919 who observed lesions in the substantia nigra, featuring loss of both pigmentation and nerve cells. The appearance of Lewy bodies in the substantia nigra and loss of neuromelanin pigment in dopaminergic neurones is still considered as histopathological characteristics of this disorder (although it is known that Lewy bodies could be absent in some genetic or early-onset forms of PD). It was, however, Carlsson whom on the basis of the behavioural and neurochemical effects of reserpine and L-DOPA on laboratory animals, suggested that PD was related to DA deficiency (Carlsson, 1959; Carlsson et al., 1957). The lack of DA in the striatum is due to degeneration of nigrostriatal neurones which originate from the *zona compacta* of the substantia nigra, but the cause of this deterioration is not yet recognized. The first symptoms appear when about 60-80% of DA nerve cells are destroyed. Utilizing [^{18}F]-DOPA uptake has assisted in monitoring the rate of progression of DA containing cell loss, but some have questioned usage of PET scans. It has been argued that this method does not correlate well with the loss of dopaminergic nerve cells but rather measure pre-synaptic AADC activity. A more explicit method is the usage of DAT tracers, indicating loss of dopaminergic cells in the brain in a more specific manner as compared to L-DOPA-based tracers (Ma et al., 2002).

The basal ganglia contain high concentrations of neurotransmitters other than DA (see Fig.1) such as 5-hydroxytryptamine (5-HT), γ-aminobutyric acid (GABA) and glutamate. Also concentrations of these neurotransmitters show discrepancies between normal and parkinsonian brains. Reduced amounts of 5-HT in the raphe nuclei and low 5-HT transporter density in the striatum has been seen in patients with PD (Doder et al., 2003; Kerenyi et al., 2003). These studies, together with a possible over expression of some 5-HT receptor subtypes in PD (suggested to be a compensatory mechanism for dopaminergic neurodegeneration) (Scholtissen et al., 2006), imply a possible degeneration of 5-HT neurones in PD. It is, however, difficult to draw any definite conclusions regarding the importance of 5-HT deficiency for the symptomatology in PD, although it is assumed to be the major cause for the depression often seen in PD patients (Leentjens, 2004).

GABA concentrations are reported to be elevated in post-mortem analysis of the striatum from PD patients (Kish et al., 1986). It is thought that this elevation, together with an increase in $GABA_A$ receptor up-regulation in the internal segment of the globus pallidus, is related to L-DOPA-induced dyskinesia (Calon et al., 2003) and not considered as a cause of disease.

The neurotransmitter glutamate also appears in high concentrations in the striatum and the substantia nigra which potentially is a risk for glutamate-induced toxicity in these brain parts. A pathological rise in extracellular glutamate may cause an un-physiological increase in the intracellular concentration of calcium ion (Ca^{2+}) through the action of N-methyl-D-aspartate (NMDA) receptors, a process called excitotoxicity. This can in turn cause excessive production of nitric oxide, mitochondrial dysfunction and finally neuronal damage (Jenner, 2003). In some studies elevated levels of glutamate have been seen in the striatum of patients with PD (Kish et al., 1986), but negative results have also been presented.

Etiology and pathophysiology

The reasons for the degeneration of DA containing neurones in PD are largely unknown; for almost all cases little is known about the etiology. In general, interaction of environmental factors with genetic constitution is believed to be the case in most patients. The origin of familial PD has been related to gene mutations, and some susceptibility genes have been determined. A number of environmental factors have been suggested to be of importance, however, a common single or cluster of factors in association with sporadic PD has not been established.

Genetics of familial PD

There are families exhibiting Mendalian pattern of inheritance of PD and responsible mutations have been identified for a few of these families. So far 11 gene loci have been linked to familial PD and they are named PARK1-11 and the gene is recognized for seven of these loci.

PARK1: The first PD gene to be discovered was the α-synuclein gene (Polymeropoulos et al., 1997). The expressed protein of this gene is found in neurones and may be important for vesicular transmitter release. α-Synuclein is the main component of Lewy bodies.

PARK2: The Parkin gene was the second PD gene to be discovered (Kitada et al., 1998). To date more than 40 mutations in this gene have been identified in families in many countries. Mutations are also found in sporadic PD cases and it has been estimated that disease appearance in 5% of all patients with onset before 50 years of age is due to Parkin gene mutations. The protein product of parkin is an E3 ubiquitin ligase, which is a component of the ubiquitin-proteosome system involved in the degradation of proteins.

PARK3: For this locus the responsible protein is not yet identified and PARK4 is a triplication of the wild-type of α-synuclein gene (Singleton et al., 2003).

PARK5: Mutation in UCH-L1, which is ubiquitin C-terminal hydroxylase L1, is linked to only one German family (Leroy et al., 1998) and the role of the gene for PD is somewhat controversial.

PARK6: Mutations in PINK 1, which is a PTEN induced putative kinase-1, was initially found in one large Italian family and recently in additionally 8 other European families (Valente et al., 2004). PINK1 is expressed in many tissues and abundantly in the CNS, and probably localised in the mitochondria.

PARK7: A DJ-1 mutation was initially found in a family in the Netherlands (Bonifati et al., 2003). In a Dutch isolate, 60 % of early onset PD could be caused by DJ-1 mutations. DJ-1 is expressed *inter alia* in the brain but its function is unknown; some data suggest that it is of importance for the cellular response to oxidative stress. Interestingly it seems to be located mainly in astrocytes.

PARK8: So far, nine mutations are known in the LRRK2 or Dardarin gene and have been found in 8 families in Europe and a group of patients in Brazilian with early onset PD (de Carvalho Aguiar et al., 2008; Nichols et al., 2007; Paisan-Ruiz et al., 2004; Zimprich et al., 2004). Mutations in this gene are considered as the most common causes of familial

PD. In Basque in Spain 5 cases of sporadic PD due to Dardarin mutations have been found and 8 % of all PD in this region can be due to Dardarin mutations. The protein is probably a cytoplasmatic kinase, phosphorylating other unknown proteins.

The phenotype for PARK9 is not typical for PD. For the PARK10 and 11 loci the responsible proteins are not yet identified.

To summarise familial PD, three genes indicate impaired protein degradation as a pathophysiological mechanism, namely α-synuclein, parkin and UCH-L1. One protein is probably involved in cellular response to oxidative stress, namely DJ-1, and two proteins are kinases.

Genetics of sporadic PD

For the majority of patients with PD, where the familial aggregation of the disease is not so evident, the genetic influence is of less importance. There is, however, evidence that genetic factors also play a significant part in the susceptibility to sporadic PD.

In a study performed in Iceland, it was shown that even for sporadic cases of PD there are familial aggregations (Sveinbjornsdottir et al., 2000). This was also reported in a community based study of PD patients with younger onset (Rocca et al., 2004). Furthermore, an epidemiological study of siblings with PD (Maher et al., 2002b) and a segregation analysis of PD (Maher et al., 2002a) indicate important genetic contribution. However, in twin studies (Tanner et al., 1999; Wirdefeldt et al., 2004) there were only a difference in concordance rate between dizygotic and monozygotic twins with an age of onset of 50 years or younger. However, when considering that the discordant twin in a twin pair could possibly acquire the disease later and hence investigate dopaminergic dysfunction using PET instead, one obtains considerably higher concordance rates for patients with an age of onset over 50 years (Piccini and Brooks, 1999). It has been suggested that the results of the epidemiological studies are compatible with a disease being caused by mutations with reduced penetrance and a late and variable age of onset (Gasser, 2005).

Many genetic polymorphic based studies have been performed on a variety of candidate genes. However, clear-cut findings that have been confirmed in several studies have been scarce. Promising is an association between a polymorphism in the interleukin 1beta gene and sporadic PD (Mattila et al., 2002; McGeer et al., 2002; Schulte et al., 2002) and a polymorphism in the glucocerebrosidase gene, coding for a lysosomal enzyme (Bras et al., 2007; Eblan et al., 2006; Sato et al., 2005).

Environmental causes

The importance of environmental causes for PD is clearly indicated by twin studies (see above). The possibility that the environment can cause, at least a PD-like syndrome, was illustrated by the patients developing PD symptoms as a result of "encephalitis lethargica" that occurred mostly in the beginning of the 20th century. Encephalitis lethargica is now hypothesised to be caused by an immune reaction to an infection by a streptococcus-like bacterium (Dale et al., 2004). The view that PD may be caused by toxins was substantiated 20 years ago, when it was reported that young addicts developed parkinsonism by taking a synthetic opioid (Langston and Ballard, 1983). It turned out that the batch contained an impurity, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The conversion of MPTP to its toxic metabolite MPP^{+} (1-methyl-4-phenyl-2,3-dihydropyridium ion) by MAO-B, the enzyme which also metabolizes DA, leads to inhibition of mitochondrial complex I and cell destruction (Nicklas et al., 1985; Nicklas et al., 1987). The findings seen following administration of MPTP to laboratory animals does not entirely show the same profile of progression and pathology as PD but this neurotoxin has been a useful tool in revealing possible mechanisms of cell death and for evaluating new therapeutic strategies (Przedborski et al., 2003). It has also been demonstrated that environmental toxins such as the insecticide rotenone or the herbicides paraquat and maneb may induce experimental parkinsonism through disturbances in the mitochondrial electron transport chain. However, the damage caused by these toxins is often not restricted to dopaminergic cells (Li et al., 2005).

In some case-control studies, but not all, an association with PD and rural living and exposure to heavy metals have been found. Well established is a negative association between PD and smoking. For reviews on PD and environmental causes see Logroscino (2005) and Brown et al. (2005). Although much experimental data support an influence of toxins acting on complex I, it is difficult to reconcile these findings with the epidemiological data showing a widespread and relatively even distribution of PD, geographically, ethnically and socioeconomically. Also, the relatively low concordance in monozygotic twins who have grown up together and the absence of concordance in married couples (Ubeda, 1998), indicate that no shared childhood or home-specific environmental factor is of fundamental importance.

Cell degeneration mechanisms

There are numerous numbers of studies giving support for various mechanisms involved in the neurodegeneration in PD. Among them are increased generation of reactive oxygen species, decreased defence against reactive oxygen species, toxins inhibiting the mitochondrial complex 1, defects in iron metabolism, excess glutamate causing an increase in intracellular calcium, and apoptosis (see Fahn and Sulzer, 2004). In recent

years several findings indicate that immune and inflammatory mechanisms are involved in the pathogenesis of PD (Liu et al., 2003), as well as impaired protein degradation as suggested by genetic linkage studies (see above). Many of these mechanisms can be a consequence of one another and neither one can be excluded. Several of these mechanisms probably can be operative simultaneously or in a time sequence.

Reactive oxygen species

The cause of degeneration of nigral DA neurones has been linked to many plausible mechanisms. One that is widely discussed and reviewed is "oxidative damage" which is the participation of reactive oxygen species (ROS), such as free radicals, in generating a hostile environment in a tissue. Free radicals are reactive atoms, ions or molecules having one or more unpaired electron which can target DNA, proteins or the lipid membrane of the cell. If the cell's ability to protect itself against these species is impaired, e.g. by down regulation of protective endogenous substances or enzymes, these reactive molecules can initiate chain reactions causing cell destruction. Molecules such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) are examples of ROS. The latter radical is the most active of these molecules and can be produced by the others during the Fenton/Haber Weiss reaction as follows:

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\bullet} + Fe^{3+} \quad \text{(Fenton)}$$
$$O_2^{\bullet -} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$
$$\overline{\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad}$$
$$O_2^{\bullet -} + H_2O_2 \rightarrow OH^- + OH^{\bullet} + O_2 \quad \text{(Haber Weiss)}$$

The metabolism of DA by MAO and aldehyde dehydrogenase to DOPAC is another source of free radical production by promoting H_2O_2 formation as follows:

$$DA + O_2 + H_2O \rightarrow DOPAC + NH_3 + H_2O_2$$

Furthermore, catabolism of DA into toxic DA-quinone species by non-enzymatic auto-oxidation shown in the following reaction is an additional source for the assembly of toxic product:

$$\text{Catecholamine (ex. DA)} + O_2 \rightarrow \text{Quinone/Semi-quinone} + H^+ + O_2^{\bullet -}$$

In the presence of superoxide, oxygen molecule is replaced by this radical and reacts with another DA molecule as follows:

$$\text{Catecholamine (ex. DA)} + O_2^{\bullet -} + 2H^+ \rightarrow (\text{semi-quinone})^{\bullet} + H_2O_2$$

Quinone derived radicals can further induce the creation of harmful molecules and augment cell degeneration (Cohen, 1994):

$$(\text{semi-quinone})^{\bullet} + O_2 \rightarrow \text{Quinone} + O_2^{\bullet -} + H_2$$

Since DA is abundant in the striatum and the substantia nigra, it is reasonable to assume that these mechanisms contribute to free radical creation and cell damage to a greater extent in these brain nuclei as compared to many other brain parts. Nitric oxide ($NO^{\bullet}$), which is itself a free radical, is also a molecule that can elevate the amount of other free radicals in a tissue and has the ability to cross membrane barriers. It can react with superoxide radical producing peroxynitrite ($^{\bullet}ONOO^{-}$). The latter can further break down and bring about a rise in the concentration of nitrogen dioxide ($NO_2^{\bullet}$) and $OH^{\bullet}$. $NO^{\bullet}$ and its derivatives have been associated with neurodegeneration and PD progression by both binding directly to DA, causing quinone formation, and by indirectly influencing different mechanism in the cell through the creation of toxic molecules (Antunes et al., 2005; Torreilles et al., 1999).

The cells defence against reactive species depends on a handful of enzymes (such as catalase and glutathione peroxidase) and molecules (e.g. glutathione, vitamin C or E), which convert these oxygen species to less toxic or harmless molecules such as water. Post-mortem studies of patients with PD show that oxidative damage may be of pathophysiological importance in this disease (Jenner, 1991, 1998, 2003; Jenner and Olanow, 1998). Increased levels of total iron and iron-(III) have been found in the substantia nigra in PD patients which can be interpreted as a consequence (Double et al., 2000; Sofic et al., 1991; Sofic et al., 1988) or cause of an increase in the production of radical species. A decrease in reduced glutathione (GSH), a molecule necessary for disintegration of H_2O_2 to water, has also been reported in the substantia nigra of parkinsonian patients (Fitzmaurice et al., 2003; Pearce et al., 1997; Sofic et al., 1992). This implies that oxidative stress can occur in this brain region due to elevated concentrations of ROS. Studies show, however, that the highest concentration of iron is not found in DA containing cells but in glia cells of patients with PD (Double et al., 2000). The same pattern has also been seen for GSH. Participation of glia cells in initiation and continuation of neurodegeneration in PD has indeed been debated because of production of different cytokines in these cells leading to oxidative stress, or their role as protective cells through the release of neurotrophic factors (Teismann and Schulz, 2004). However, resent studies suggest that changes in glutathione concentrations are not specific for PD (Fitzmaurice et al., 2003) and that the observed alterations can merely be an effect of aging. It has been difficult to establish if ROS are a primary cause to the findings seen in PD or if it is other alterations in the cells which bring about a rise in the production of reactive molecules (Jenner 1991; 1998 and 2003).

Current treatments

Although at present no cure exists for PD, symptomatic and palliative treatment can improve the patient's quality of life (Schapira, 2007). Dopamine replacement therapy using L-DOPA is still considered as the most effective treatment. It is used in combination with a peripheral decarboxylase inhibitor (e.g. carbidopa or benserazide) in order to minimize the transformation of L-DOPA to DA outside the brain. Motor related adverse effects, such as "wearing off" (the return of motor deficit symptoms at the end of the dosing interval) or the "on-off" phenomenon (experiencing unpredictable rapid fluctuation in motor activity and performance) are unwanted complications believed to be associated with the progression of the disease and the long-term discontinuous L-DOPA administration (Obeso et al., 2000). DA agonists such as apomorphine, bromocriptine and cabergoline; MAO-B inhibitors such as selegiline and rasigiline and finally COMT inhibitors such as entacapone and tolcapone are also important drugs in the treatment of PD. Trials of surgical management have also been performed with few advantages compared to drug treatment (Jankovic, 2006), demanding access to special operative centres and surgeons. Deep brain stimulation (DBS), however promising, is not applicable on all patients nor without risk or side effects (Limousin and Martinez-Torres, 2008). Likewise, stem cell transplantation offers a source of cell substitution in neurodegenerative diseases (Wang et al., 2007), but a constant debate on ethical issues has been heard and evaluations have over all shown small therapeutic effects and also troublesome side effects (Hagell and Cenci, 2005). These methods put forward encouraging prospects but need much improvement in order to replace pharmacotherapy. The major consideration in dealing with affected patients is to find the best suitable therapy according to the need of each individual patient.

CYTOCHROME P450 ENZYMES

General characteristics

The P450-containing monooxygenase system has been divided into two main types: bacterial/mitochondrial (type I) and microsomal (type II). The CYP450 type II enzymes are part of a microsomal mixed function oxidase (MMFO) system which catalyse the incorporation of one oxygen atom into their substrate. The name is given because of the enzyme's property of having a spectral absorbance maximum at 450 nm. These enzymes are 45-55 kDa in size, having a haem moiety with iron protoporfyrin IX as their prosthetic group. They have existed for more than 1.4 billion years and now include 78 families in 155 species (CYP 450 data base at *http://cpd.ibmh.msk.su*). Each family (defined by an Arabic number following CYP or 450 (e.g. CYP2) exhibits an amino acid sequence similarity of 40 percent or more and is divided into subfamilies (defined by a letter following the Arabic number, e.g. CYP2E) which display a minimum of 55 percent

sequence identity. This is followed by another Arabic number denoting the specific gene/protein (e.g. CYP2E1). In the vertebrate families of CYP450, families 1 to 3 are the major hepatic catabolic enzymes responsible for the phase I metabolism of a wide variety of endogenous and exogenous substances, showing overlapping substrate specificity. Some enzymes in these families are also active in some extra hepatic tissue such as kidney, lung and brain (Bhamre et al., 1992; Farin and Omiecinski, 1993; Kapitulnik and Strobel, 1999; Seliskar and Rozman, 2007). Genetic polymorphism is seen in all CYP450 families, predominantly in families 1 to 3 which display a relatively low evolutionary genetic preservation (Ingelman-Sundberg, 2004).

CYP450 enzymes are membrane bound proteins (bacterial enzymes excluded). In hepatocytes, the largest fraction of the enzyme is located at the membrane of the endoplasmatic reticulum (ER) (Loeper et al., 1993; Neve and Ingelman-Sundberg, 2000) oriented mainly towards the cytoplasm but also towards the lumen (Neve and Ingelman-Sundberg, 2000). In addition they are found in the Golgi apparatus and at the plasma membrane (Bar-Nun et al., 1980; Neve et al., 1996; Stasiecki et al., 1980). Determining their three dimensional structure by crystallography has not been an easy task but the few microbial, rabbit and human CYP450 enzymes which structures have been revealed provide valuable information about the activity of these enzymes (Johnson, 2003). A few functional domains common for all CYP450 have been identified (Gonzalez, 1988; Johnson, 2003). These are the hydrophobic membrane insertion segment at the amino terminal and the haem-binding domain close to the carboxy terminal. A site for the binding of electron donors (e.g. NADPH-cytochrome P450 reductase) is also a conserved part of the enzyme's structure.

The apoprotein and the haem moiety of the CYP450 enzyme are synthesized separately and the assembly of the two parts occurs by a complex route (Gibson and Skett, 1994). As many other enzymes, the CYP450 enzymes can be affected by endogenous and exogenous influences leading to induction, inhibition, down-regulation or merely degradation of the holoenzyme. Regulation of these enzymes can occur at all levels i.e. transcriptional, post-transcriptional, translational or post-translational. For example, in the former cases, activation of gene expression of CYP2E1 has been seen only within a few hours after birth (proposed to be coupled to activation of transcription factor HNF-1 and demethylation of cytosine residues upstream of the transcription start site) and at the onset of puberty (Gonzalez, 1988; Gonzalez and Gelboin, 1990; Johnson, 2003; Vieira et al., 1996). The post-transcriptional up-regulation of these enzymes occurs by mRNA stabilization, translation enhancement or enzyme stabilization (Gonzalez, 1988) such as after ethanol administration. These are caused by covalent/non-covalent alterations (Aguiar et al., 2005). It is proposed that down-regulation of the enzymes by posttranslational modification of the apoprotein or the haem moiety is due to formation of active transient species such as ROS, epoxides or ketones from so called suicide

substrates, e.g. cyclopropans, alkyls or aromatic amines (Karuzina and Archakov, 1994b). Ubiquitination or inactivation of the haem moiety through complex formation with NO is also considered as a cause of down-regulation (Aguiar et al., 2005; Correia et al., 2005). Tyrosine nitration of the apoprotein by means of reaction with peroxynitrile derived from NO is another route leading to degradation (Aguiar et al., 2005).

Catalytic cycle

The MMFO system consists of a CYP450 enzyme, the electron donor NADPH cytochrome P450 reductase (with some exceptions) and a lipid component (phosphatidylcholine). This system needs an oxygen molecule and NADPH in order to insert an oxygen atom into the substrate. The catalytic reaction can be simplified as follows:

$$\text{Substrate-}\mathbf{H} + O_2 + \text{NADPH} + H^+ \xrightarrow{\text{CYP 450}} \text{Substrate-}\mathbf{OH} + H_2O + NADP^+$$

The electron donor NADPH cytochrome P450 reductase is a flavoprotein consisting of both a flavin mononucleotide (FMN) and a flavin adenine dinucleotide (FAD) moiety. The latter attains two electrons from NADPH which are then transferred one at a time to the FMN moiety, resulting in an initial semiquinone and finally fully reduced form of this functional group (Coon, 2005). However, it has been noted that CYP450 enzymes can utilize other electron donors such as NADH-cytochrome b_5 reductase via cytochrome b_5 (Aoyama et al., 1990; Truan et al., 1993). Some microbial CYP450 enzymes have even demonstrated a fusion system with their electron donors, creating a more efficient electron transfer to their catalytic centre (McLean et al., 2005). Nevertheless NADPH reductase is considered as the general electron donor in the MMFO system and has been located in the same compartments as CYP450 in rat liver cells (Neve et al., 1996; Stasiecki et al., 1980). This enzyme has also been found in microsomes of whole rat brain and cortical human microsomes using Western immunoblotting (Anandatheerthavarada et al., 1992).

The catalytic cycle of CYP450 is schematically shown in Fig. 3. With the binding of substrate, the enzyme's redox potential is altered due to a change in the spin state of the ferric haem iron. This facilitates the first electron transfer to the enzyme and hence the conversion of ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron. The next step is the binding of dioxygen to Fe^{2+}, generating an instable complex which undergoes electron rearrangement to yield a ferric oxy species (Fe^{3+}-O_2^{-}). The delivery of the second electron to the enzyme and creation of the peroxy complex (Fe^{3+}-O_2^{2-}) is followed by production of a water molecule and formation of an iron-oxene species ($(Fe{=}O)^{3+}$). The final step is the insertion of an oxygen atom (this step has been proposed to involve the formation of a

substrate radical as an intermediate). The product is then disconnected and CYP450 is returned to its initial state.

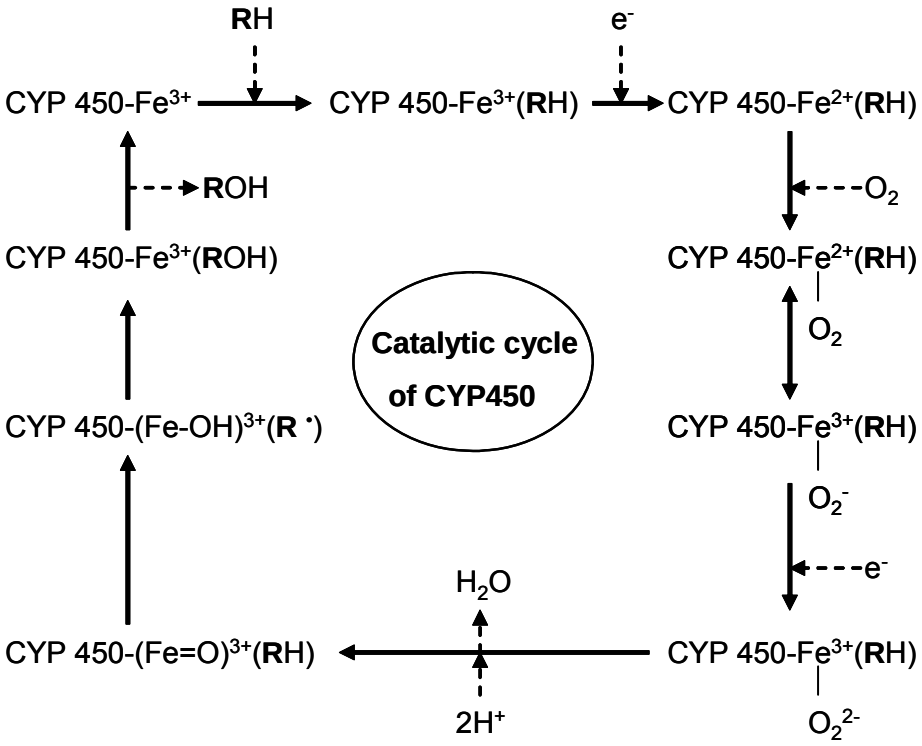

Figure 3. *Schematic display of the catalytic cycle of cytochrome P450. Abbreviations: R, substrate; CYP450, cytochrome P450 apoprotein.*

Cytochrome P450 2E1

CYP2E1 has been found in many extrahepatic organs, for example in kidney and lung. However, the liver demonstrates the highest concentration of the enzyme (de Waziers et al., 1990; Thomas et al., 1987). CYP2E1 has many substrates and products, some of which are generally toxic or carcinogenic. Its active site pocket is relatively small and hydrophobic, creating a suitable environment for small non-polar molecules. Among the many endogenous substrates of the enzyme that have been identified (Ingelman-Sundberg, 2004; Lieber, 1997; Ronis et al, 1996), are ketones (e.g. acetone) and fatty acids such as arachidonic acid. The exogenous compounds metabolized by CYP2E1 comprise a wide variety of xenobiotics (e.g. acetaminophen, aniline, paracetamol, N-nitrosodimetylamine and chlorzoxazone, with the latter two often used as enzymatic probes), alcohols (e.g. ethanol, methanol), acetaldehyde, aromatic hydrocarbons such as benzene and toluene, halogenated hydrocarbons (e.g. carbon tetrachloride) and finally

anaesthetics including enflurane, isoflurane and halothane. A list of a total of 160 substrates, 102 inhibitors and 28 inducers of the enzyme are available at CYP450 data base at *http://cpd.ibmh.msk.su*. The K_m values for most of these substrates are within the nM limit, showing a high affinity for the enzyme.

With the detection of active CYP450 in microsomes from whole rat brain, as seen by spectral absorbance peak measurements and oxidation of estradiol (Cohn et al., 1977; Sasame et al., 1977), it was realized that also the brain may have CYP enzyme activity and by that the potential to metabolise and detoxify toxic exogenous substances. It became subsequently apparent that many CYP450 enzymes are present in brain tissue, including CYP2E1. Hansson et al. (1990) detected this enzyme in neurones and glia cells of rat brain, in many nuclei including the substantia nigra. It was later shown that CYP2E1 in rat brain cells of the basal ganglia, including substantia nigra and striatum, also could be induced by ethanol or nicotine administration (Anandatheerthavarada et al., 1993a; Anandatheerthavarada et al., 1993b; Sohda et al., 1993). At the same time, CYP2E1 mRNA expression was demonstrated in diverse parts of the human brain, including substantia nigra (Farin and Omiecinski, 1993). Shortly after, the enzyme was found in the substantia nigra pars compacta of rat, within cells that morphologically resembled dopaminergic neurones and was shown to be induced in striatal blood vessels by isoniazid, a known inducer of CYP2E1 (Riedl et al., 1996). Also, induction of this enzyme during ischemic injury was shown in hippocampal and cortex astrocytes of rat and gerbil *in vivo* (Tindberg et al., 1996). Watts et al. (1998) showed that inducible CYP2E1 existed in the same compartment as tyrosine hydroxylase in the rat substantia nigra but could not detect the enzyme in nigral glia cells. In addition, localisation of the enzyme in monkey brain, as well as prenatal and adult human brain was confirmed (Brzezinski et al., 1999; Joshi and Tyndale, 2006b; Upadhya et al., 2000). CYP2E1 has been found in an active form in ER (microsomes), the Golgi apparatus and the plasma membrane of rat hepatocytes (Loeper et al., 1993; Neve et al., 1996; Wu and Cederbaum, 1992). It is possible that in the CNS, the active form of this enzyme is localized in the same membrane compartments as its hepatic variety.

Factors influencing CYP2E1 expression and activity

As other CYP450 enzymes, regulation of CYP2E1 takes place at all levels from gene expression to post-translation and can vary between species, tissue, agent and administration technique. Enzyme induction occurs by diverse endogenous and exogenous influences such as starvation, diabetes or various substances and is due to effects on the path of enzyme expression as early as transcription, but also through later modifications such as by way of post-translation. Also, the same substance can cause induction at different levels of expression as in case of ethanol administration (Table 1.). Regulation of CYP2E1 by xenobiotics in form of transcriptional induction is however not

common. Inhibition of this enzyme can be caused by metabolites or intermediates of suicide substrates. It has been shown that CYP2E1 displays a biphasic half-life. This is illustrated in a pattern consisting of a rapid phase half-life of 6-7 hours followed by a slower phase of approximately 37 hours in the rat (Roberts et al., 1994; Song et al., 1989), with the domination of the slow phase half-life in the presence of ethanol/acetone. However, a longer degradation half-life of 50 hours or more has been shown for this enzyme in healthy humans (Emery et al., 1999). The reason for this biphasic kinetic disappearance is not yet clear. It could be due to a possible degradation of the enzyme being initiated by two different routes, namely destruction of the haem moiety (causing rapid obliteration) or the apoprotein (causing slower obliteration) (see above). There is also the possibility that lysosomal based degradation or substrate stabilization, is responsible for the slower phase, and ubiquitin-dependent/-independent proteosome-based degradation (as in the case for many membrane bound proteins) executes the rapid phase (for detailed discussion see Gonzalez, 2007). Microsomal ubiqutin-conjugates have been observed as a product of CYP2E1 break down in rat liver microsomes after suicide inactivation by agents such as carbon tetrachloride (Tierney et al., 1992), confirming that this enzyme is subjected to proteosomal degradation. Its elimination by a lysosomal route has also been proven (Ronis et al., 1991). This has been coupled to haem loss by the appearance of only the apoprotein in lysosomal fractions. Song et al. (1989) suggested the existence of two populations of CYP450, one localized in smooth and another present in rough ER, leading to decrease in translation and loss of the enzyme by diverse paths. A selection of some of the most studied factors causing induction, inhibition or down-regulation of this enzyme is given in the table below (Table 1.). It is important to emphasise that both endo- and exogenous factors play a part in influencing CYP2E1 activity and availability.

Table 1. *Selected summery of some endo- and exogenous factors influencing CYP 2E1. Vertical arrows represent rise/fall of following feature.*

Influencing factor	Effect	Reference
Birth	- Gene activation by transcription: ↑protein, ↑ RNA level	(Vieira et al., 1996)
Ethanol	- at low concentrations: protein stabilization - at high concentrations: mRNA stabilization	(Cederbaum, 2006) (Lieber, 1997)
Acetone	- in rat liver microsomes: ↑ protein stabilization, ↑activity	(Song et al., 1989) (Tierney et al., 1992)

Table 1. Continued

Triiodothyronine (T_3)	- in rat liver: ↑ protein expression, ↑ activity - rabbit CYP 2E1 measured in human HepG2 cell line: ↑ protein expression, ↑ mRNA t½	(Fernandez et al., 2003) (Oinonen et al., 1996) (Peng and Coon, 1998)
Starvation/diabetes	- in rat: ↑ protein level, ↑ mRNA	(Hong et al., 1987) (Song et al., 1987)
Acetylsalicylates	- in rat blood, liver microsomes: ↑activity, ↑ transcription, ↑ protein synthesis - in human HepG2 cell line, cultured rat hepatocytes: ↑protein level, ↓enzyme turnover	(Pankow et al., 1994) (Damme et al., 1996) (Wu and Cederbaum, 2001)
Interleukin-4	- in human hepatocytes, HepG2 cell line: ↑mRNA	(Abdel-Razzak et al., 2004)
Lipopolysacharide, Interleukin-1β	- in cultured rat astrocyte, rat and gerbil brain: ↑ mRNA, ↑ protein activity	(Tindberg et al., 1996)
Nicotine	- acute administration in rat brain: regional specific rise in protein level - chronic administration in rat, monkey brain: general rise in protein level	(Joshi and Tyndale, 2006a, b)
Isoniazid	- in rat liver microsomes: ↑ mRNA translation - healthy subjects, pharmacokinetic models: ligand-stabilization	(Park et al., 1993) (Zand et al., 1993) (Chien et al., 1997)
Insulin	- in cultured rat hepatocytes: ↓ transcription, ↓ mRNA t½	(Woodcroft and Novak, 1997)
Phenylethyl-isothyocyanate	- in rat liver microsomes: ↓ protein level, ↓activity, ↓ mRNA	(Ishizaki et al., 1990) (Lindros et al., 1995)
Dialylsulphide	- suicide inhibition	(Brady et al., 1991a) (Yang et al., 2001)
Disulfiram (Diethydithiocarbamate)	- in rat microsomes: ↓ protein activity - healthy subjects: ↓activity (↓ chlorzoxazone clearance)	(Brady et al., 1991b) (Kharasch et al., 1993)
Interleukin (IL)-1β, IL-6, TNFα, IFNγ	- in human hepatocytes, HepG2 cell line: ↓mRNA, ↓ protein activity	(Abdel-Razzak et al., 2004; Abdel-Razzak et al., 1993)

Catalytic cycle of CYP 2E1: uncoupling and ROS production

It has been proposed that CYP2E1 is responsible for the production of active metabolites that are carcinogenic or toxic (see below). Such toxicity has in some cases been associated with the production of free radical intermediates. Although CYP450 enzymes demonstrate a common catalytic cycle, it has been shown that for some of these enzymes, e.g. CYP2E1, this cycle can be disrupted by various substrates, so called "uncouplers" (Staudt et al., 1974), underlying this toxicity. In these cases, the enzyme-substrate complex is formed, but assimilation of the oxygen atom into the substrate is not accomplished because of sterical or chemical obstruction. This, occurs above all during the catalytic cycle of CYP2E1, inducing a discharge of ROS in form of $O_2^{\bullet -}$ created in the enzyme-substrate complex, followed by its dismutation and conversion to H_2O_2 and $OH^{\bullet}$ (Ekstrom and Ingelman-Sundberg, 1989; Ingelman-Sundberg and Johansson, 1984; Persson et al., 1990). This process has been observed in microsomal liver samples from rat and also in rabbit reconstituted membrane vesicle systems. As a consequence, lipid peroxidation can be initiated by the enzyme (Ekstrom and Ingelman-Sundberg, 1984, 1989) and has been confirmed through inhibition of this process by CYP2E1 polyclonal antibodies. ROS production and lipid peroxidation products (polyunsaturated fatty acids), sensitive to CYP2E1 antibody addition have also been detected in astrocyte cultures after treatment with ethanol (Montoliu et al., 1995).

Another outcome of uncouplers is oxidative inactivation of the enzyme or so called suicide inhibition (Karuzina and Archakov, 1994a, b), where destructive substrate intermediates produced by the disintegration of the peroxy complex covalently bind to the haem group (as seen in activation of amine substrates by this enzyme) or the apoprotein. In all the above mentioned situations ROS is produced, either as $O_2^{\bullet -}$ (by oxy complex decay) or as H_2O_2 (by peroxy complex decay or dismutation of $O_2^{\bullet -}$). The binding of substrate and the uncoupling caused by the leakage of the substrate-peroxy complex is believed to be the main cause of autoinactivation, producing H_2O_2 at the catalytic centre of the CYP450 enzymes (Karuzina and Archakov 1994a).

It is important to state that the production of ROS by CYP2E1 can even be observed in the absence of exogenous substrates (Ingelman-Sundberg and Johanson, 1984) and even during presence of inducers (as seen in ethanol-treated astrocyte cultures), and not only inhibitors (Montoliu et al., 1995). Taken together, it is evident that CYP2E1 activity can under certain circumstances be potentially cell toxic through the production of ROS, although, it is plausible that the cell's protective mechanisms to remove excess ROS produced by CYP2E1 is normally adequate or adapt to the increased ROS formation, as shown in a HepG2 cell line expressing this enzyme (Mari and Cederbaum, 2001). Elevated levels of microsomal glutathione-S-transferase and catalase activities were

specifically seen in this cell line compared to control cell culture, indicating a plausible increase of H_2O_2 in the CYP2E1 expressing cells.

Cytochrome P450 enzymes and Parkinson's disease

Earlier studies brought about the idea that CYP enzymes may play a role in the pathophysiology of PD as they are detoxifying enzymes and therefore contributing to the metabolism of environmental toxins and at the same time demonstrating polymorphic varieties (Barbeau et al., 1985; Ferrari et al., 1986; Ho et al., 1996; Shahi et al., 1990). Studies have given opposing results as to the significance of these enzymes in the susceptibility to PD. A general summery of relevance to PD and in order of quantity of reported investigations is given below.

Cytochrome P450 2D6 (CYP2D6)

Debrisoquine 4-hydroxylase or CYP2D6 is an enzyme participating in the metabolism of MPTP to toxic/non-toxic products (Herraiz et al., 2006) and has been found *inter alia* in the substantia nigra of the human brain (Gilham et al., 1997). It has also been suggested to be involved in the formation of DA from tyramine (Hiroi et al., 1998). Initial reports implied that poor metabolising genotypes, not possessing the functional form of CYP2D6, were seen in a higher proportion in patients with PD and those with early onset of the disease as compared to control subjects (Barbeau et al., 1985; Smith et al., 1992). A case-control study, also considering history of solvent exposure, found an association between individuals with this disease and poor metabolising variants of CYP2D6 only when combined with solvent exposure (De Palma et al., 1998), but failed to show any association to the disorder *per se*. Other reports have failed to confirm any association (Nicholl et al., 1999; Sabbagh et al., 1999), however, exposure to environmental toxins was not considered in these studies. An increased risk of developing PD due to combination of poor metabolising capacity and pesticide exposure was confirmed in a more recent study showing an approximately two-fold risk of increase compared to non-poor metabolisers (Elbaz et al., 2004). Nevertheless, this interaction, as in previous studies, was only seen in a small group of patience. This result was repeated by an additional investigation (Deng et al., 2004), but a recent study failed to show any association between two poor-/extensive activity alleles of the enzyme and PD (Bialecka et al., 2007). Altogether, more than twenty studies investigate the possible involvement of CYP2D6 in neurodegenerative disorders but illustrate contradicting results (Riedl et al., 1999). This enzyme has approximately twenty five allelic variants and based on existing outcomes it is presently difficult to draw any definite conclusions regarding its role as a susceptibility gene for PD.

Cytochrome P450 1A1 (CYP1A1)

CYP1A1 (7-ethoxyresorufin O-deethylase) metabolises a variety of polycyclic aromatic hydrocarbons and can be induced in the lung by nicotine (Daly et al., 1993; Iba et al., 1998; Kim et al., 2004). It has been proposed that smoking has a protective effect against PD (Gorell et al., 1999; Morens et al., 1995) which raised the interest to inspect polymorphic forms of this enzyme in patients with this disease. In addition, one study demonstrated that nicotine treatment inhibited the immunoreactivity of CYP1A1 and CYP1A2 in the striatum of rat (Anandatheerthavarada et al., 1993b), drawing more attention to the activity of these enzymes in relation to PD. A study based on a Japanese population found an association between this disorder and an allele variant of CYP1A1, that is related to increased enzyme activity (Takakubo et al., 1996). In the same study the relative risk for PD was higher among subjects with the homozygote form of a mutation causing an amino acid substitution as compared to subjects with the homozygote wild type. This association could, however, not be confirmed by a Chinese investigation (Chan et al., 2002). In the latter study, regional dissimilarities concerning exposure of toxic chemicals and a possible linkage disequilibrium of this gene with the nearby gene of CYP1A2 was discussed and given as a possible cause for this discrepancy between the two studies. Although evidence for an association of these enzymes with PD is not convincing, the possible bioactivation of specific toxic compounds by these enzymes in the liver, which in turn participate to the degeneration of neurones in the basal ganglia can not be totally ruled out. Further investigations are needed in order to clarify the role of these CYP450 enzymes in the brain.

Cytochrome P450 2C9 (CYP2C9) and Cytochrome P450 2C19 (CYP2C19)

The genotypes of poor metabolisers of CYP2C9 (S-Warfarin 7-hydroxylase) and CYP2C19 (S-Mephenytoin 4-hydroxylase), which are responsible for parahydroxylation of phenytoin and metabolism of S-mephenytoin respectively, have been investigated in small groups of individuals with PD. CYP2C19 is known to metabolise a number of psychoactive drugs (Coutts and Urichuk, 1999). The metabolising activity of the latter enzyme, based on genotype and excretion of a CYP2C19 metabolite, illustrated no significant discrepancy between PD patients and control subjects in two independent studies. The first survey (Gudjonsson et al., 1990) was performed in a Swedish population based on hydroxylation of S-mephenytoin but lacked power due to the small number of individuals studied (34 patients). The second analysis examined the genotype frequencies in Japanese populations among which 63 were diagnosed with PD (Tsuneoka et al., 1996). Again, the small number of patients created a low power in this study. A group of investigators (Ferrari et al., 1990) examined the possibility that poor metabolising ability of phenyotoin by CYP2C9 is involved in the pathogenesis of PD and showed that significantly more PD patients than controls were intermediate or poor

metabolizers, but no correlation between metabolic ratio of the enzyme and age of onset or duration of the disease was found. However, the number of individuals in the studied groups was very small. This finding could not be confirmed by a later study performed on patients having young-onset of the disease (Peeters et al., 1994). The evidence of any association between these enzymes and PD is altogether not convincing. This could nevertheless be a consequence of the undersized groups analysed.

Cytochrome P450 2E1

A few studies and findings indicate that CYP2E1 may be of relevance for PD pathophysiology. In one study, inhibition of the enzyme by three different inhibitors caused a rise in the extracellular DA concentration in the substantia nigra but not in the striatum, as measured by microdialysis in rat (Nissbrandt et al., 2001). The highest increase of DA was observed during inhibition by phenylethyl isothiocyanate (PEITC). Furthermore, an association between the enzyme and MPTP has been presented (Vaglini et al., 2004; Viaggi et al., 2006). An augmentation of MPTP toxicity, evaluated by tyrosine hydroxylase immunoreactivity, was seen in the substantia nigra pars compacta during CYP2E1 inhibition in mice. Furthermore, DA contents of wild-type mice also indicated enhanced toxicity after MPTP treatment combined with a CYP2E1 inhibitor, whereas CYP2E1 knockout animals did not demonstrate this escalation. Parkinsonism induced by n-hexane (Pezzoli et al., 1995) is another possibility of participation of CYP2E1 in this neurodegenerative disorder through the metabolism of this solvent to the toxin 2,5-hexandione (Iba et al., 2000).

The human CYP2E1 protein consists of 493 amino acids. The gene is located on chromosome 10 and consists of a classic TATA box and nine exons (Umeno et al., 1988). The approximately 12 kb sequenced base pairs have since then been extensively investigated for possible polymorphisms and related functions (The International HapMap Project at *http://www.hapmap.org*; Home page of the Human Cytochrome P450 Allele Nomenclatur Committee at *http://www.cypalleles.ki.se*). Some studies have been able to relate functional features of the enzyme to polymorphic forms. One study showed that CYP2E1 inducers such as alcohol or morbid obesity in human subjects, together with an approximately 100-bp insertion mutation in the 5´-flanking region of the enzyme, increased the chlorzoxazone metabolism measured as the concentration of this probe's metabolite (6-hydroxychlorzoxazone) in plasma (McCarver et al., 1998). Two other studies (Hanioka et al., 2003; Hu et al., 1997) have in an expression system using COS-1 cells, demonstrated that Arg76His substitution due to a mutation in the coding region of the enzyme (creating CYP2E1*2 polymorphic form, nomenclature according to Home page of the Human Cytochrome P450 Allele Nomenclatur Committee), caused a reduction in protein concentration compared to wild type CYP2E1. This polymorphic variety also exhibited reduced activity determined by chlorzoxazone 6-hydroxylation and

4-nitrophenol 2-hydroxylation. It is therefore possible that polymorphic diversity of this enzyme can be associated with different functional qualities and hence possible diseased state.

The participation of CYP2E1 in activation of pro-carcinogens such as aniline, N-nitrosodimethylamine and so forth has connected this enzyme's activity to malignancies in various organs, examples are association of CYP2E1*5B or CYP2E1*6 polymorphisms with esophageal, nasopharymgeal, gastric, lung and liver cancer (Danko and Chaschin, 2005). However in these cases, discrepancies in genotype distribution frequencies in different ethnic populations have generated conflicting results. Much attention has been given to a possible role of CYP2E1 in initiating alcoholic liver disease (ALD). This is due to localization of the enzyme in the centrilobular zone of the liver, where most damage is seen during ALD, together with the enzyme's contribution to the metabolism of ethanol to acetaldehyde or 1-hydroxyethyl radical (Cederbaum, 2006). Despite certain induction of this enzyme by ethanol, no conclusive results as to association of polymorphic forms of CYP2E1 and ALD have yet been established.

There have only been limited inquiries concerning CYP2E1 polymorphisms and PD, investigating only the Pst I and Rsa I restriction site polymorphisms at the 5'-flanking region. Three studies, on a European population (Bandmann et al., 1997), a Taiwanese (Wang et al., 2000) and a Chinese population (Wu et al., 2002), all failed to show any association between PD and these polymorphisms.

OBJECTIVE OF THE STUDY

Previous investigations have indicated that CYP2E1 inhibition increases extra cellular DA in the substantia nigra. One main aim of this thesis was to elucidate the mechanisms responsible for this increase and to explore possible involvement of CYP2E1 in DA metabolism and neurotransmission. It is also known that the enzyme can generate ROS which can contribute to the neurodegenerative processes in PD. Therefore, a further goal was to conduct a preliminary examination concerning the participation of this enzyme in producing these species in the substantia nigra.

The CYP2E1 gene expresses polymorphism and therefore it was of interest to inspect polymorphic variants in a population of patients with PD and in control subjects with the purpose to find possible association with this disease. A final intention was to examine whether different splicing variants of the CYP2E1 gene exist in some human brain regions that are of relevance for PD.

METHODOLOGY

Studies on the effect of CYP2E1 inhibition on DA metabolism and release (*Papers I and II*)

Enquiry regarding *in vivo* DA metabolism was based on a novel approach. This comprised the injection of a tritiated DA-precursor (L-DOPA) to rats (*Paper I*), followed by biochemical post-mortem analysis of the supernatant obtained from relevant brain regions. High performance reversed-phase ion-pair chromatography (HPLC) and a scintillation system were used for separation and measurement of radioactivity in supernatant fractions.

The study concerning release of DA (*Paper II*) was executed utilizing *in vivo* microdialysis technique. The mechanisms responsible for the increase in extracellular DA induced by CYP2E1 inhibition were investigated by measuring the effects of drugs interfering with different processes involved in DA release during CYP2E1 inhibition. The concentration of DA and its metabolites in microdialysate were detected by HPLC.

Free radical production was also determined using microdialysis technique and was measured by the transformation of the trapping agent 4-hydroxybenzoic acid (4-HB) to 3, 4-dihydroxybenzoic acid (3, 4-HB) during local perfusion through the microdialysis tubing, followed by measurement in an HPLC system (*Paper II*).

Ethics

The experimental designs were approved by the local ethical committee in Gothenburg and animal procedures were performed in accordance with the European Communities Council Directive of 24 November 1986. All efforts were made to minimize the number of animals used and their suffering.

Animals

The choice of animal for pharmacological studies is important regarding the possibility to correctly extrapolate the results to human physiology and pathophysiology. One point is ethical considerations. At the same time, the issue studied should show functional resemblance between the model species and human. The rat and mouse have been predominant models of choice for neuropharmacological research. In addition, in these animals the CYP2E1 gene shows approximately 80% similarity to its human counterpart. Conducting microdialysis experiments on mice, in such small brain nucleus as the substantia nigra, is however not without difficulties. This is the main reason why rat

became the selected species in the present *in vivo* experiments. Furthermore, the rat is considered as the best replica of choice for studying activity and function of CYP2E1 gene and relating the findings to outcomes in the human (Martignoni et al., 2006).

The animals used for *in vivo* experiments were male Sprague Dawley rats (B&K Universal AB, Sollentuna, Sweden) weighing 250 g on arrival. They were kept 2-5 per cage for one week prior to experiment initiation and under controlled environmental conditions (temperature 26°C; humidity 60-65%, light 5 a.m. - 7 p.m. and dark 7 p.m. - 5 a.m.). In studies based on the microdialysis technique (*Paper II*), each animal was housed in a separate cage following probe implantation. Food and tap water were available *ad lib*. The animals weighed 250-300 g on the day of experiments. All experiments were conducted during day time.

Drugs

Radioactive dopamine precursor:

Radiolabelled L-DOPA [L-3,4-(ring 2,5,6-^{3}H) dihydroxyphenylalanine] (specific activity ≈50 Ci/mmol, concentration 1 mCi/ml) (American Radiolabeled Chemicals Inc., St. Louis, MO, USA)

Vesicular amine transporter inhibitor:

Reserpine (Ciba-Geigy, Basel, Switzerland)

Peripheral COMT inhibitor:

Entacapone [OR-611; N,N-diethyl-2-cyano-3-(3,4-dihydrozy-5-nitrophenyl) acrylaminde] (Orion Pharma, Espoo, Finland)

Peripheral AADC inhibitor:

Carbidopa [MK 486; L-2-hydrazino-3-(3,4-dihydroxyphenyl)-2-methyl-propionic acid] (Merck Sharp & Dohme International Rahway, NJ, USA)

Inhibitor of firing of dopaminergic neuron:

γ-butyrolactone (GBL) (Sigma-Aldrich AB, Sweden)

Dopamine transporter inhibitor:

1, 4-dialkylpiperazine (GBR-12909, Research Biochemical Incorporated, Natick, MA,USA)

Drugs used for detection of reactive oxygen species:

3, 4-dihydroxybenzoic acid (3,4-HB) (Sigma-Aldrich AB, Sweden)

4-hydroxybenzoic acid (4-HB) (Sigma-Aldrich AB, Sweden)

Cytochrome P450 2E1 inhibitor:

Phenylethyl isothiocyanate (PEITC) (Acros Chimica N.V., Geel, Belgium)

Voltage sensitive sodium channel blocker:

Tetrodotoxin (TTX) (Sigma-Aldrich AB, Sweden)

Analgesic:

Ketoprofen (Romefen® Vet, Merial, Lyon, France)

Anaesthetics:

Isoflurane (Forene®, Abbot Scandinavia AB, Solna, Sweden)
Chloral hydrate (KEBO Lab AB, Stockholm, Sweden)
Ketamine (Parke-Davis, SA, Barcelona Spain)
Xylazine (Bayer, Leverkusen, Germany)

Brain dissection techniques and tissue preparation

In experiments performed for quantification of radioactivity (*Paper I*), the rats were sacrificed by an injection of chloral hydrate (400 mg/kg, i.p.) followed by decapitation. The brains were quickly removed and placed on an ice-chilled petri dish. The striatum was dissected as performed by Carlsson and Lindqvist (1973) and the substantia nigra was isolated according to Nissbrandt et al. (1985). After dissection the tissues were immediately placed on dry ice and then stored in a freezer at -70° until the time of their analysis. The average weights of the striatums and the substantia nigras were about 80 mg and 8 mg, respectively.

The dissected tissues were homogenized in 0.1 M $HClO_4$, 10 mM Na_2EDTA and 3 mM reduced glutathione by an ultrasonic disrupter (Sonifier, Type B-30). Striatum and substantia nigra were homogenized in 1.06 and 0.35 ml, respectively. After centrifugation (≈ 8 000 x g, 0° C, for 10 min), 200 µl of the supernatant was taken for injection into a reversed-phase ion-pair HPLC system.

In the study utilizing microdialysis technique (*Paper II*), brain tissue was dissected out in order to verify the position of the implanted probe. Whole brain tissue was removed from the sacrificed animals and sliced with a vibratome. Probe traces were detected macroscopically and excluded from the study in case of significant haemorrhage or inaccurate position.

Treatment schedule for radioactivity quantification (Paper I)

To minimize extracerebral catabolism of L-DOPA all animals were given the peripheral DOPA decarboxylase inhibitor, carbidopa (20 mg/kg, s.c., Bartholini and Pletscher, 1969) and the peripheral COMT inhibitor, entacapone (30 mg/kg, s.c., Törnwall et al., 1994). Thirty min later the rats received an i.v. injection of tritiated L-DOPA (0.06 mCi; the rats were placed inside a rat restrainer and the solution was injected into a tail vein) and were sacrificed 30, 60 or 90 min thereafter. The rats sacrificed 60 min after L-DOPA received either PEITC (100 mg/kg, i.p.) or vehicle 20 min before they were sacrificed.

Biochemical analysis of supernatant (Paper I)

The chromatography system analysing the supernatants consisted of a HPLC-pump (LKB 2150, Pharmacia LKB Biotechnology Sverige AB, Sollentuna, Sweden), a stainless steel column (150 x 4.6 mm, luna 5µ C18, Phenomenex Torrance, CA, USA) and an amperometric detector (Waters 460, Millipore Waters, Milford, MA, USA) with a glassy carbon working electrode operated at 0.75 V versus Ag/AgCl. An integrator (Spectra Physics SP 4270, San José, CA, USA) monitored the resulting current. The mobile phase consisted of 0.0125 M K_2HPO_4, 0.0375 M citric acid, 0.053 mM Na_2EDTA, 0.26 mM octyl-sulfate sodium salt and 8% methanol (pH ≈ 3). The flow rate was 1.1 ml/min. The external standard used for identifying peaks contained 50 ng/ml of each of the following compounds: DOPA, DOPAC, DA, HVA, 3-methoxytyramine (3-MT), 3,4-dihydroxymandelic acid (DOMA), 3,4-dihydroxyphenylglycol (DOPEG), 3-methoxy-4-hydroxy-mandelic acid (VMA), norepinephrine (NE), 3-methoxy-4-hydroxy-phenylglycol (MOPEG), normetanephrine (NM), α-methylDOPA (α-MD), 3-O-methyl-DOPA (3-OMD), 5-hydroxyindoleacetic acid (5HIAA), 5-hydroxytryptamine (serotonin, 5-HT) and 3-methoxy-4-hydroxyphenylethanol (MOPET) (all compounds from Sigma-Aldrich Sweden AB, Stockholm, Sweden).

Quantification of radioactivity (Paper I)

Collection of fractions in scintillation vials (POLY Q-vials, Wallac OY, Turku, Finland) started immediately after injection of the supernatant into the HPLC-system. The dead volume between the analytical cell and scintillation vials was negligible (less than 5 µl). Fractions were gathered manually after their passage through the analytical cell at two-minute intervals, with the exception of the DOPA, DA, DOPAC, HVA and 3-MT peaks, which were each separately, collected from start to finish of their peaks. In every experiment separate volumes (50 µl) of homogenate and supernatant were also taken for scintillation analysis. Scintillation counting was based on a 2 ml volume from each fraction in a 10 ml Ready Safe scintillation cocktail (Beckman Instruments Inc., Fullerton, CA, USA). Mobile phase was added to those fractions, which did not have the required volume, obtaining the total volume of 2 ml. The mobile phase was also used as blank in the system (2 ml mobile phase + 10 ml cocktail). The amount of radioactivity, disintegration per minute (DPM), of each fraction was then measured in a liquid scintillation counter (LS 6500 scintillation system, Beckman Instruments Inc., Fullerton, CA, USA) in a sequence of three separate cycles. A schematic illustration of the investigation performed in *Paper I* is given in Fig. 4.

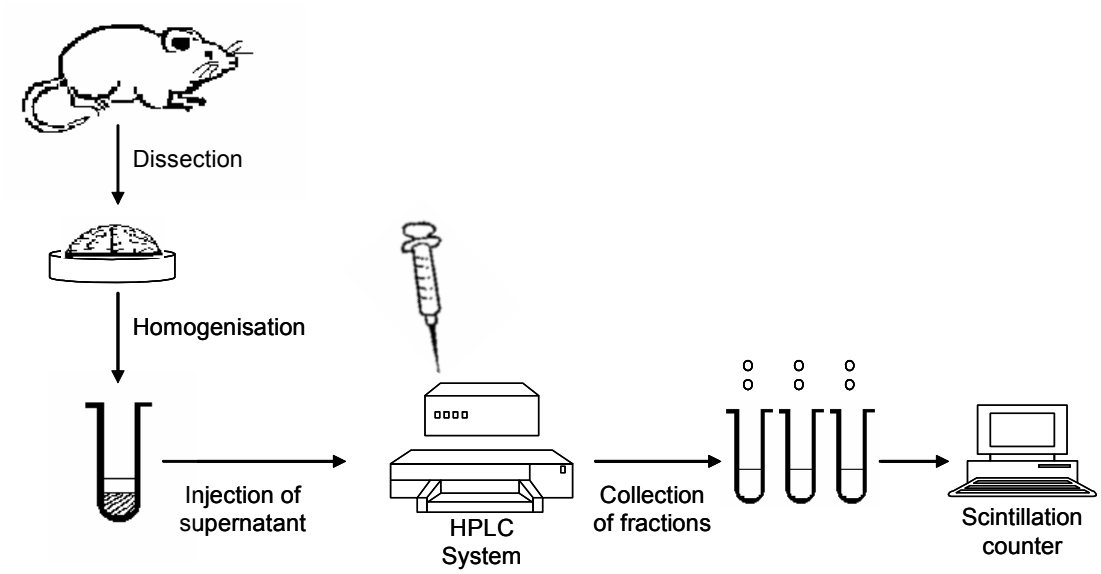

Figure 4. *A schematic drawing of the experimental procedure for radioactivity measurements of the supernatant of dissected tissue.*

Microdialysis, surgical implantation of probe and performance (Paper II)

Microdialysis technique in the brain is based on utilizing the concentration gradient between the interstitial space and a perfusing solution. This is made possible by a semi permeable membrane, allowing the passage of water and small molecules while a continuous flow of the perfusion fluid is maintained. Diffusion of molecules through the membrane into the perfusion fluid permits the analysis of molecules in the collected dialysate, representing extracellular concentrations in the brain. In order to minimize contamination in the dialysate caused by a damaged blood brain barrier (BBB), it is necessary to allow the tissue to recover after implantation of the membrane and the connected in/outlet tubing (so called microdialysis probe). It has been shown that BBB regains an intact form roughly 30 min - few hours after probe implantation as measured by the concentrations of the amino acid α-aminoisobutyrate in rabbits. Approximately 24 hours following surgery, the concentration of this amino acid has shown a satisfactory correlation to the corresponding cerebrospinal fluid concentrations (CSF) (for a more detailed review on micorodialysis see Benveniste, 1989 and Benveniste and Hüttemeier, 1990). There are however some downsides in using microdialysis which are first and foremost attaining the balance between a reasonable concentration measurement (without an unnatural rise in transmitter concentration due to damaged tissue) and avoiding gliosis (caused by prolonged presence of the implanted probe). There is also the difficulty of accurate surgery in smaller nuclei of the brain. The method is also time-consuming, but

altogether is considered as one of the best suitable methods of date for estimating the extra cellular concentrations of neurotransmitters *in vivo*.

During probe implantation, isoflurane anaesthesia (delivered by a Univentor 400 Anaesthesia Unit, Univentor Ltd., Zejtun, Malta) was administered to maintain surgical anaesthesia as determined by loss of tail-pinch and corneal reflex reactions. The anesthetized animals were placed in a stereotaxic frame with a horizontal plane through lambda and bregma. Microdialysis I-shaped probes, produced at our laboratory (described previously by Elverfors et al., 1997) with 20-kD cut off membranes (AN69HF membrane, Filtral 16; Hospal Ind., Meyzien, France) and possessing an exposed length of 2mm were used. Prior to implantation, the probes were perfused with ethanol (70%) followed by Ringer solution (see below for chemical composition) and sealed by heating. Probe implantation in the substantia nigra was performed using the following coordinates from bregma according to Paxinos and Watson (1986): A/P -5.3, L/M -2.3 and V/D -8.6. The animals received an immediate postoperative dose of ketoprofen (5 mg/kg, s.c.) for analgesia. All animals received a plastic collar for later attachment to the swivelled perfusion arm and were allowed to recover in separate cages for approximately 40 hours after which the microdialysis experiments were conducted. For the evaluation of free radical production, animals were anaesthetized either with isoflurane, which is a CYP2E1 substrate, or a mixture of ketamine (90 mg/kg) and xylazine (20 mg/kg) as the opposing anaesthetic. Extra fluid in form of 4 x 1.0 ml 0.9% NaCl (s.c.) was administered if surgery was performed using ketamin-xylazine anaesthesia. Probe implantation and general microdialysis technique were identical with the procedures performed in the experiments on DA and DA metabolites.

On the day of experiment the inlet of the probe was connected to a perfusion pump (CMA/100; Carnegie Medicine, Sweden) and the outlet tube was attached to collecting plastic vials via the swivel perfusion system. The probes were then perfused with modified Ringer solution (140 mM NaCl, 1.2 mM $CaCl_2$, 3.0 mM KCl, 1.0 mM $MgCl_2$, according to Moghaddam and Bunney, 1989) at a rate of 2.1 µl /min and allowed to equilibrate for one hour; henceforth basal values were obtained in three consecutive samples. A volume of 42 µl dialysate was collected for each 20 min sample and was immediately analyzed in the HPLC-system. Dialysate samples of volume of 52.5 µl were collected during 25 min for analysis of free radical production.

Treatment schedule during microdialysis performance (Paper II)

Directly after attainment of baseline values the animal was injected with a drug or appropriate vehicle according to the experimental procedures and further samples were collected under the remaining time of the experiment. In experiments conducted with reserpine, GBR-12909 and GBL, drug injection was followed 120, 80 and 40 min,

respectively, by administration of PEITC (100 mg/kg, i.p.) or vehicle. In the tetrodotoxin experiment 1 µM of the drug was perfused through the microdialysis tubing after baseline dialysate collection. For the evaluation of free radical production, the perfusion of 20 µM 4-HB dissolved in the modified Ringer solution, was started immediately after baseline (first three samples) dialysate collection. This perfusion proceeded for a period of 75 min and was replaced by modified Ringer solution after discontinuation.

Biochemical analysis of dialysate (Paper II)

An HPLC-system with electrochemical detection was used to analyse the dialysate samples. The samples were analyzed in a split fraction system developed by Lagerkvist (1999). The system consisted of a cation-exchange HPLC column for detection of DA and a reverse-phase system for its metabolites DOPAC and HVA. After sample collection, each vial was immediately placed in a refrigerated microinjector (CMA/200, CMA Microdialysis AB, Solna, Sweden) containing two sample loops. A sample volume of 26 µl was injected into the cation-exchange column (Nucleosil 5µ SA 100A, 150 x 2.0 mm Phenomenex, Torrance, CA, USA) , with a mobile phase consisting of 0.049 M citric acid, 0.0114 M NaOH, 0.012 mM Na_2EDTA and 20% methanol (pH = 5.3-5.5). The reverse phase column (Nucleosil 3µ C_{18} 100A, 2.0 x 50 mm, mM Na_2EDTA) was injected with 11 µl of dialysate. The mobile phase for this detection system contained 0.010 M K_2HPO_4, 0.040 M citric acid, 0.012 mM Na_2EDTA, and 5% methanol (pH = 2.8-2.9). An amperometric detector was used for the detection of DA (Decade detector, Antec Leyden, Leiden, Netherlands) which operated at 0.45 V versus an Ag/AgCl reference electrode. Another amperometric detector (Waters 460, Millipore Waters, Milford, MA, USA), was operated at 0.8 V versus an Ag/AgCl reference electrode and used to detect DOPAC and HVA. The currents were integrated with a chromatography software package (Dionex Chromeleon, Dionex, Sunnyvale, CA, USA) on a Windows NT PC platform. An external standard containing 3.26 nM DA, 297 nM DOPAC and 274 nM HVA was used. The detection level of DA was three times the noise level (~ 0.02 - 0.07 nM = 0.02 - 0.07 fmol/µL dialysate).

Free radical measurement analysis (Paper II)

The HPLC-system used for the detection of free radicals (3, 4-HB) contained a reversed phase column (Synergi 4µ Hydro-RP 80A New Column 250 x 4.6 mm, Phenomenex, Torrance, CA, USA) together with a pre-column (C18 4 x 3.0 mm 10/Pkg, Security Guard Cartridge, Phenomenex, Torrance, CA, USA) and a similar amperometric detector as used for the DA detection (see biochemical analysis of dialysate in *Paper II*), operating at + 0.7 V. The mobile phase was composed of 0.010 M K_2HPO_4, 0.040 M citric acid, 0.053 mM Na_2EDTA, 0.11 mM octyl-sulphate sodium salt and 13.5% methanol (pH = 2.44). The resulting current was monitored by an integrator (Spectra

Physics SP 4270, San José, CA, USA). Dialysate volume of 50 µl was injected into a Rheodyne 7725 injector. An external standard of 5 nM 3, 4-HB was used.

Considerations regarding free radical detection

Using 4-HB as the trapping agent has both advantages and disadvantages (Chen and Stenken, 2002; Liu et al., 2002; Marklund et al., 2001; Ste-Marie et al., 1996; Ste-Marie et al., 1999). The advantages are: a) 4-HB is a better $^{\bullet}OH$ trapping agent compared to salicylate (2-hydroxy-benzoic acid) because there is only one hydroxylated output (3, 4-HB) created, avoiding splitting of the signal into two or more products, b) 3, 4-HB is a stable product which can be measured by HPLC or alternative methods, c) the auto-oxidation of 4-HB is less pronounced than that of other salicylates, and finally d) 4-HB can penetrate the blood brain barrier by means of an acidic transporter implying that it also can enter the brain after systemic administration. In some pilot studies we administered 4-HB systematically (up to 400mg/kg, i.p.) and were able to detect its product to some extent in the dialysate (unpublished data). Disadvantages with 4-HB are similar as for any other trapping agent used together with the microdialysis technique. The major points to consider are that exposure to light, air, metal or plastic surfaces can cause a spontaneous production of 3, 4-HB. This is however more pronounced when high concentrations of 4-HB (~ 1mM) are used. To reduce spontaneous unspecific production of 3, 4-HB, we minimized the exposure of the 4-HB solution to light, used glass syringes, shorter dialysis tubing and utilised a relatively low concentration of the compound (20 µM). To avoid formation of hydroxylated of 4-HB by peroxynitrous acid (ONOOH), produced by interaction between increased NO caused by isoflurane (Baumane et al., 2002) and superoxide ($O_2^{\bullet -}$) from damaged tissue, the tissue was allowed to recover for 48 hours after probe implantation.

Genetic studies (*Papers III and IV*)

A polymorphism-based study was conducted in *Paper III*. A polymorphism is a genetically determined variation which affects a minimum of 1-2% of the population under study. These heritable DNA changes permit the occurrence of genetic diversity in a population. In this paper, the CYP2E1 gene was examined in a control population and a population with PD using a tag-single nucleotide polymorphism (tSNP) approach founded on HapMap (The International HapMap consortium, 2003) data. A tSNP is a single nucleotide polymorphism (SNP) which represents a non-haphazard and frequent association of alleles at different loci i.e. linkage disequilibrium (LD). This allows investigating polymorphic variations of the genome with high LD, without having to genotype all SNPs in the studied chromosomal region and facilitates the search for genotype-disease connection. Six t-SNPs were used in the analysis in *Paper III*, and

haplotype block (a set of inherited and associated SNPs in near location and on the same chromosome) data was obtained. In case of significance, the SNP was further examined regarding early/late age of disease onset and possession of relatives with PD. The analyses were carried out using TaqMan technology or pyrosequencing method.

In *Paper IV*, the possibility of existence of alternatively spliced variants of CYP2E1 was investigated in five putamen and two caudate nuclei obtained from five deceased individuals. Total RNA was extracted from these tissues and converted to cDNA. Thereafter, CYP2E1 cDNA was amplified by a polymerase chain reaction (PCR) and subjected to gel electrophoresis, exposing the spliced isoforms.

Ethics

All participants in the study conducted in *Paper III* were included after giving their informed consent and the study was approved by the ethical committees at University of Gothenburg and Karolinska Institute. The study performed in *Paper IV* was approved by the ethical committee at University of Gothenburg.

Cohorts

PD Patients in *Paper III* were diagnosed according to the "Brain Bank clinical diagnostic criteria" (Daniel and Lees, 1993) except that presence of more than one relative with PD was not an exclusion criterion. The patients were recruited from Sahlgrenska University Hospital in Gothenburg, Karolinska University Hospital in Stockholm and hospitals in Skövde and Falköping, Sweden. The control subjects in *Paper III* were individuals visiting hospitals and care centres in Gothenburg and Stockholm. Additional information concerning possible first/second/third degree relatives with PD and age at the time of blood donation were recorded for both patients and controls when possible. Furthermore, age of disease onset noted as the beginning of symptoms was obtained from the PD patients. The demographic data for patients and controls are summarised in Table 1 in *Paper III.* The studied individuals in *Paper IV* were of European origin and had passed away at 65-83 years of age, during the years 1988-1989 at Sahlgrenska University Hospital. None of the individuals had any sign of neurological/neurodegenerative disease.

Genomic extraction and techniques

For genomic DNA extraction in *Paper III*, blood samples were immediately frozen after withdrawal. Extraction was carried out by a ML STAR instrument (Hamilton Robotics, USA), using the AGOWA® mag Maxi DNA isolation Kit Plus (AGOWA GmbH, Germany). In order to cover most of the genetic variability of the CYP2E1 gene, six

tSNPs were chosen according to implications by the HapMap project (*http://www.hapmap.org/*) using pairwise tagging, r^2 cutoff ≥ 0.8, Mean Allele Frequency ≥ 0.1 and population CEU (Utah residents with ancestry from northern and western Europe). For details concerning the chosen SNPs see *Paper III*.

Total RNA (*Paper IV*) from the right putamen of five deceased individuals was extracted using the RNeasy Lipid Tissue Kit (QIAGEN Inc., USA). Furthermore, total RNA of the caudate nucleus of two of these individuals was also purified using the same procedure. This kit is designed for the best possible extraction of RNA from tissues rich in fat, such as brain. SuperScript™ II Reverse Transcriptase Kit (Invitrogen Corporation, USA) was used to synthesize cDNA, utilizing 1µg of the purified RNA and 0.5 µg Oligo(dt) primers. Samples without addition of superscript reverse transcriptase (-RT) during preparation of cDNA were considered as negative controls. The normalization of CYP2E1 cDNA was carried out using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

TaqMan® single nucleotide polymorphism assay

TaqMan® method is founded on Real-time polymerase chain reaction (Real-time PCR) implementation and resembles standard PCR method with the exception that a double-labelled fluorogenic probe (TaqMan probe) is used (De la Vega et al., 2005). The probe is a single stranded oligonucleotide (>20 nucleotides) complementary to a segment of the DNA which includes the selected SNP and anneals to the template between the two primers. It contains a fluorescent reporter (flurophore) molecule and a quencher at its 5' and 3' ends respectively. As DNA synthesis proceeds reaching the bound probe, degeneration of the probe is executed by the exonuclease activity of the Taq polymerase releasing the flurophore from the molecule and hence from the quencher, allowing detection of fluorescence. A cluster plot is produced and interpreted for all the samples. In this way, allelic discrimination is achieved following amplification in "real time". An illustration of TaqMan method is given in Fig. 5.

In *Paper III,* genotyping was performed (with the exception for one SNP) by 5'nuclease allelic discrimination TaqMan assays containing fluorescently (VIC/FAM) labelled probe/primer sets for selected SNPs, purchased from Applied Biosystems Inc., Foster City, USA. All assays were carried out in accordance to the manufacturer's recommendations utilizing TaqMan technology and the ABI 7900 Sequence Detection System. Genotypes were analyzed by the SDS v 2.2.1 software (Applied Biosystems Inc., Foster City, USA).

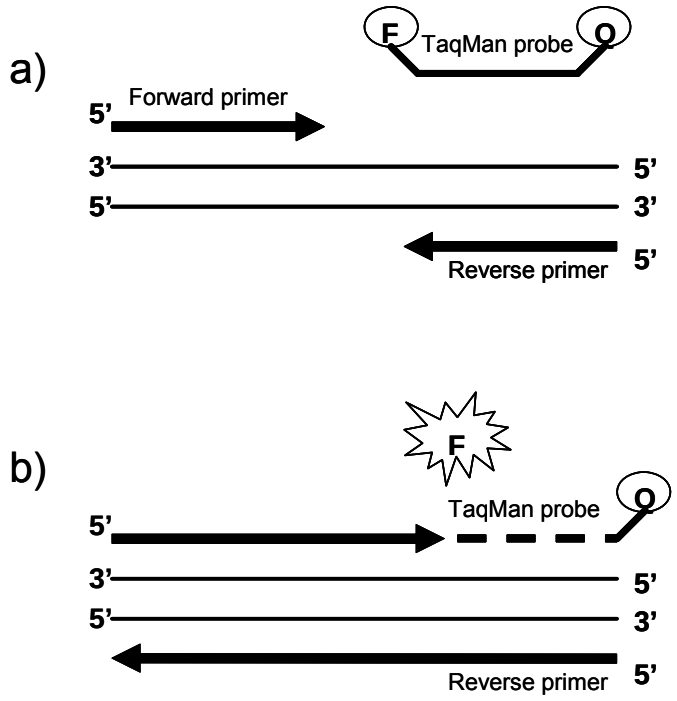

Figure 5. *An illustration of the TaqMan method utilizing a TaqMan probe. a) The TaqMan probe and the template prior to extension. b) The flurophore is released during extension. Abbreviations: F, flurophore; Q, quencher.*

Pyrosequencing™

Due to the low rate of determined genotype for one analysed SNP exploiting the TaqMan assay, the analysis of this SNP was carried out using the pyrosequencing method (Biotage, UppsalaSweden) according to this supplier's instructions.

The pyrosequencing method is initiated with adding of different nucleotides (dXTPs), one at a time, to the single stranded DNA by the act of DNA polymerase (Fakhrai-Rad et al., 2002). This occurs subsequent to binding of a short sequenced primer to the template in the vicinity of the SNP in question. Incorporation of the dXTP leads to the release of pyrophosphate (PPi), which is converted to adenosine-5'-triphosphate (ATP) through the action of ATP-sulfurylase. Thereafter, a luciferin molecule is converted to oxyluceferin by luciferase, utilizing the produced ATP. Energy in form of a light signal is emitted and detected during this last reaction, portrayed as a peak in a pyrogram™. In order to avoid consumption of dATP by luciferase and causing misleading signals, deoxyadenosine alpha-thio triphosphate (dATPαS) is used as a substitute. Degradation of excess dXTP

and ATP is carried out by the enzyme apyrase prior to addition of the next dXTP. Hence, an absence of signal is apparent in case of a non-corresponding dXTP against the studied SNP. A schematic illustration of pyrosequencing is given below in Fig. 6.

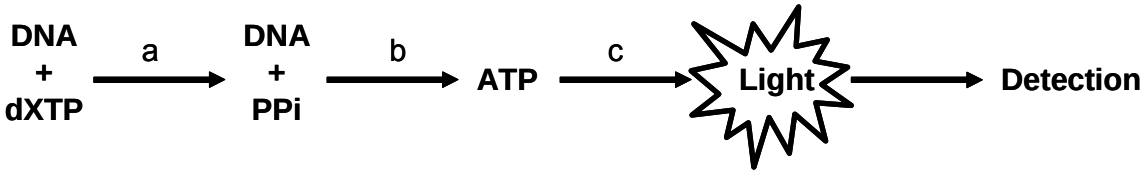

Figure 6. *A schematic illustration of the Pyrosequencing™ method. The enzymes catalysing the different stages are: a, DNA Polymerase; b, ATP Sulfurylase; c, Luciferase. Abbreviations: dXTP, a deoxynucleoside triphosphate; PPi, pyrophosphate, ATP, adenosine-5'-triphosphate.*

Statistical methods

Paper I:

- Student's t-test was used for evaluation of differences of total radioactivity of homogenised tissues between nuclei.
- Differences between corresponding fractions of tissue samples from the two brain nuclei were statistically analysed by calculating confidence intervals on the differences between values from the two nuclei for each rat, in the respective supernatant fraction. Bonferroni adjustment was applied to all confidence intervals.
- Differences between controls and treated samples for each tissue were statistically evaluated by two-way ANOVA followed by Bonferroni adjustment.

Paper II:

- One-Way Analysis of Variance (One-Way ANOVA) followed by Bonferroni post hoc test was used for comparison between treatment and baseline values in experiments performed using PEITC, reserpine and TTX. In the experiments with reserpine the concentration of DA were at some time points below the detection limit and therefore replaced by a value corresponding to this limit in order to allow performance of statistical calculations.
- The same statistical method as above was used to estimate the effect of GBR-12909, GBL and 4-HB, where treated and untreated animals were compared.

Paper III:

- Deviation from Hardy-Weinberg equilibrium was calculated by Chi square (χ^2) test.
- Haplotype block estimation for evaluation of differences between patients and controls was conducted using Haploview software and a permutation test procedure consisting of 100 000 permutations was applied.
- Fisher's exact test was performed for comparing allele frequencies between patients and controls.
- In case of significant association, χ^2 test was used for genotype analysis between these groups and also for further statistical analysis of the significant SNP considering age of onset and recorded first/second/third degree relatives with PD.

P> 0.05 was regarded as the significant level in all performed statistical analyses.

RESULTS

Studies on dopamine metabolism and release

In the first part of the thesis, post-mortem analysis of dissected rat brain tissue, with or without the inhibition of CYP2E1 was conducted. This was executed after administration of radioactive L-DOPA with the purpose to reveal possible effects of CYP2E1 inhibition on the pattern of DA metabolism in the striatum and substantia nigra. This initial study was followed by an in vivo microdialysis examination of DA release in the substantia nigra, in which the effects of a CYP2E1 inhibitor combined with various pharmacological tools known to affect DA neurotransmission were analysed. In addition, a preliminary investigation of ROS production was undertaken.

In ***Paper I*** a new method was developed to generally widen the search for alternative metabolites of DA, and more specifically to study the effects of CYP2E1 inhibition on DA metabolism. The method is based on systemic administration of tritiated L-DOPA and subsequent radioactivity measurements of reversed-phase ion-pair HPLC-separated fractions derived from dissected brain tissue. It was possible to identify and separate fractions containing the major catecholamines and their metabolites. By optimising the chromatography conditions a total separation of the substances L-DOPA, DOPAC, DA, HVA, 3-MT, NM, α-MD, 3-OMD, 5HIAA and 5-HT could be achieved. The amount of radioactivity, in the different fractions varied considerably. The fraction that contained DA showed the highest proportional amount of radioactivity, and being significantly

higher in the substantia nigra than in the striatum. Relatively high radioactivity was also observed in the L-DOPA and DOPAC fractions, but radioactivity was low in the HVA and 3-MT-fractions. Moderate radioactivity was observed, especially in the substantia nigra, in the first three 2-min fractions. The last two of these fractions contained NE and its metabolites DOMA, DOPEG, VMA and MOPEG. The radioactivities of three fractions, where no known metabolites are localised, were also significantly higher in the substantia nigra than in the striatum. These were the fraction after L-DOPA and the fraction before HVA (at 30 and 60 min, respectively) and the first 0-2 min fraction (at all time intervals). The relative distribution of radioactivity did, however, not significantly differ between the three time intervals in either brain region, except for the fraction containing L-DOPA showing a progressive decline with time in both brain parts. In addition, when total homogenate radioactivity was normalised to tissue weight, no significant difference was seen between the two brain structures or during the time course that was investigated for each tissue.

Treatment with PEITC increased the relative radioactivity in the second 2-min fraction from both nuclei (see Fig. 3, ***Paper I***). There was, however, no difference in the total homogenate radioactivity/tissue weight between the controls from either tissue and PEITC treated rats (measured 60 min after L-DOPA administration). The radioactivity of the L-DOPA, DA, DOPAC, HVA and 3-MT fractions were similar in controls and in PEITC treated rats and were not included in the statistical analysis because they were not relevant for the tested hypothesis (alternative DA metabolites).

In order to elucidate the mechanisms responsible for the increase in extracellular DA concentration in the substantia nigra induced by CYP2E1 inhibition, the effects of the CYP2E1 inhibitor PEITC on extracellular DA in rat, monitored by *in vivo* microdialysis, was analysed following administration of pharmacological tools interfering with different aspects of dopaminergic neurotransmission, i.e. GBR 12909 (inhibition of DA reuptake), γ-butyrolactone (inhibition of firing rate of DA neurones), tetrodotoxin (inhibition of voltage dependent sodium channels) and reserpine (inhibition of DA storage) (***Paper II***). The administration of PEITC (100 mg/kg, i.p.) induced a robust increase in extracellular DA, as measured by microdialysis, peaking at ~ 200 % of the basal value and confirming a previous investigation (Nissbrandt et al., 2001). The addition of PEITC to GBR-12909 pre-treated animals (4 mg/kg, s.c.), also induced a rise in DA, peaking at ~ 350% of the basal value. Prior to PEITC administration a gradual increase of nigral extracellular DA concentration by maximally ~ 95 % was observed following GBR-12909 treatment. GBL treatment (750 mg/kg, i.p.) induced a clear-cut decrease of the nigral extracellular DA concentration to 30-50 % of the basal value. When PEITC was given to these animals DA increased rapidly, reaching around 250% of the basal value. Local perfusion of 1 μM of TTX induced a rapid decrease of measured DA concentration to minimally 13 %. No further significant effect on extracellular DA concentration was seen in these animals

after injection of PEITC. Systemic administration of reserpine (5 mg/kg, s.c.) also caused a rapid decline of the DA concentration in the substantia nigra below the detection limit and additional treatment with PEITC, 120 minutes following reserpine administration, did not raise the DA concentration above this limit.

Experiments investigating the effect of isoflurane on free radical production demonstrated an apparent distinction between this method of anaesthesia and the combined ketamine (90mg/kg, i.p.) and xylazine (20mg/kg, i.p.) procedure. In animals anaesthetized by the former drugs during probe implantation surgery, no significant increase in dialysate 3, 4-HB concentration could be seen following local 4-HB (20μM) administration. However, in animals anaesthetized by isoflurane, an increase was observed (see Fig. 6, ***Paper II***). The 3, 4-HB concentration increased immediately after 4-HB administration and reached a peak of approximately 750% of basal value, which declined after discontinuation of 4-HB.

To ascertain the nature of the analysed peak corresponding to 3, 4-HB, it was necessary to carry out random measurements of both 4-HB (20μM) and 3, 4-HB (5nM, according to the employed standard solution) through injection of these compounds into the HPLC system. This was done in order to exclude the possibility that the peak equivalent to the trapped free radical is merely measured 4-HB or caused by spontaneous transformation of this substance to 3, 4-HB in the chromatography system used. Chromatograms of injected 4-HB and 3, 4-HB are shown in Fig. 7.

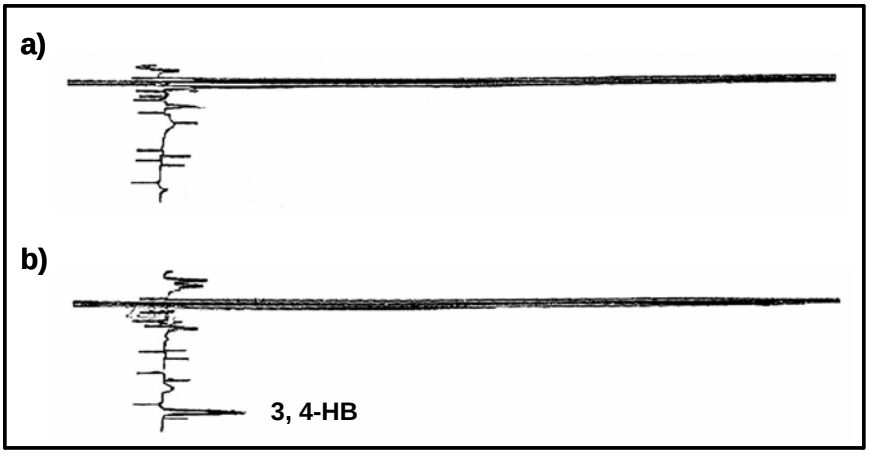

Figure 7. *Chromatogram of injected compound into the HPLC system utilized during free radical measurements. a) Chromatogram of injected 4-HB (20μM) and b) Chromatogram of injected 3, 4-HB (5nM), demonstrating a peak corresponding to the retention time for this substance according to a standard solution.*

Injection of 20μM 4-HB did not create a quantifiable peak during any occasion of random analysis and hence could not disturb or augment 3, 4-HB measurements, whereas a peak caused by injection of 5nM 3, 4-HB was apparent (Fig. 7). These random examinations were also performed for testing of the microdialysis probe, tubing and connected swivelled perfusion system when not attached to animals but perfused with 4-HB. No noticeable peak caused by 4-HB or production of its metabolite was ever recorded utilizing 20μM 4-HB. Conversely, an increase in size of the peak constituting of 3, 4-HB was evident during mircrodialysis sample measurements subsequent to perfusion of 20μM 4-HB into the animals (Fig. 8).

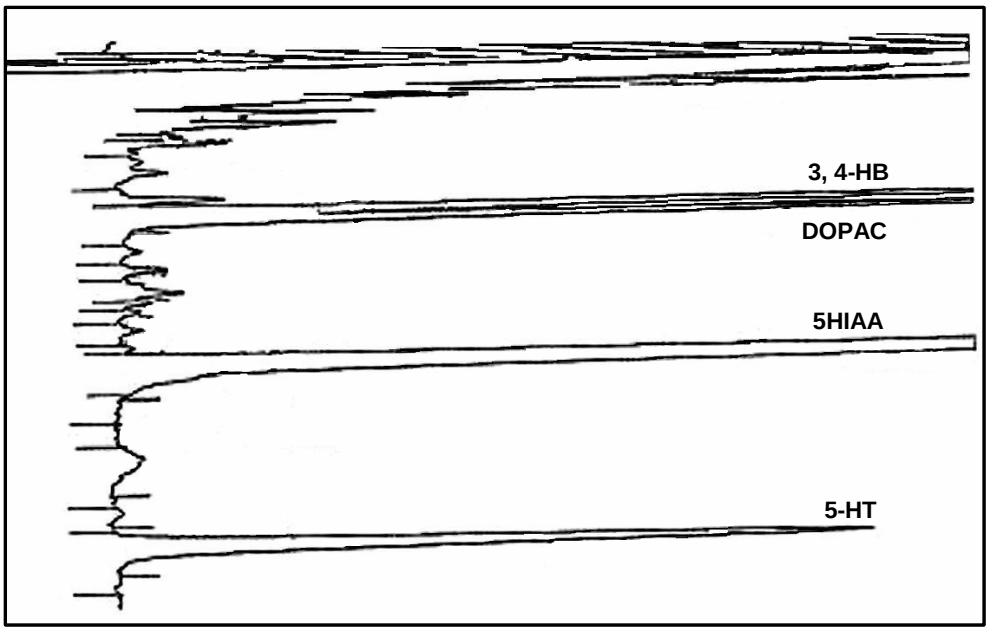

Figure 8. *Chromatogram of a microdialysis sample, 75 min subsequent to perfusion start with 20μM 4-HB. A much noticeable rise in the concentration of 3, 4-HB is evident.*

Together, the results in ***Papers I*** and ***II*** indicate that CYP2E1 inhibition induced by PEITC alters the metabolic pattern of L-DOPA and that the increase in extracellular DA is critically dependent on reserpine-sensitive stored DA and voltage dependent sodium channels.

Studies on genetic variations of cytochrome P450 2E1

The genotypes for all investigated polymorphisms among the control population were distributed according to the Hardy-Weinberg ($P > 0.01$) equilibrium. Allele and genotype frequency comparisons between PD patients and control subjects demonstrated significant differences for one of the studied polymorphisms, namely a C/G

polymorphism positioned at intron 7. This SNP displayed higher frequency of the C allele in PD patients. The same polymorphism was further analyzed regarding age of disease onset, illustrating the same pattern of association for both genotype and allele frequencies in PD patients with an age of onset later than fifty years of age. However, no significance was obtained analyzing this polymorphism regarding earlier disease onset. The same single marker also showed a significant relation in genotype, allele and haplotype frequencies (the haplotype association was, however, not significant following permutation test) while comparing PD patients not having relatives with PD with controls, illustrating the higher frequency of the C allele in patients. PD patients having a relative affected with PD showed no significant association for this marker in comparison to controls (for details on obtained significances see ***Paper III***).

Investigation of putamen of the five individuals in ***Paper IV*** revealed two possible variants of amplified CYP2E1 cDNA. The appearance of a dominant band characterizes a molecular weight higher than the 1114 bp of the molecular ladder which could correspond to the unspliced (wild type) form of the enzyme having 1228 bp. This form of CYP2E1 is seen in all the five inspected putamen nuclei. The other apparent band in these nuclei is a weaker luminescence just below 1114 bp, presumably representing the spliced form consistent with a CYP2E1 spliced alternative (GenBank mRNA sequence: AJ853939) in NCBI-Ace View (at *http.//www.ncbi.nlm.nih.gov/*). The two caudate nuclei examined also showed the unspliced wild type of CYP2E1. In addition, in the latter brain part of one of these individuals, the same alternative spliced variant as seen in the putamen was distinguished.

Results from ***Papers III*** *and* ***IV*** indicate a CYP2E1 gene polymorphism association with PD and demonstrate diverse spliced variations in human brain.

DISCUSSION

Studies on dopamine metabolism and release

Radioactive probe

The choice of exploiting L-DOPA with a tritiated ring as the radioactive probe was grounded on several factors (***Paper I***). These were availability of the compound, uptake into the CNS (which ruled out DA as a probe), high and specific detection (reduced in case of employing tyrosine) and reduction of non-specific labelling caused by

rearrangement or loss of ^{3}H. Considering the latter matter, assuring the continuity of radioactivity through the steps leading to DA and its metabolites could be strengthened through the use of L-DOPA with a tritiated ring. Moreover, a putative novel metabolite is more likely to consist of the roughly unchanged aromatic part of DA. Applying 6-[18F]fluro-DOPA in this study was not preferred due to some differences in the pharmacokinetics properties of this substance and endogenous L-DOPA. Metabolites of fluro-DOPA posses more lipophilic characteristics than the endogenous substances and this can alter the retention time in the HPLC system. Moreover, due to a higher extent of acidity, this substance has more affinity for COMT and phenol-sulfotransferase, and in a comparative study on *in vivo* metabolism of tritiated- and fluro-DOPA, the former contributed in a higher degree to the synthesis of DA in the striatum (Melega et al., 1990). We are nevertheless aware that the 6-position label is a potential location for a nucleophilic attack during oxidation of catecholamines and also sulfoconjugation. This could be a source of radioactivity from non-DA related molecules. However, this "unspecific" radioactivity does not necessarily have to be in the front fractions, where the highest DPM values were registered.

Distribution of radioactivity

The overall pattern of radioactivity suggests a very rapid initial uptake of L-DOPA after i.v. administration and a rapid conversion to DA in both nuclei. It is also apparent that the DOPAC radioactivities are much higher than the HVA radioactivities and indication of a decrease in DOPAC and an increase in HVA is seen with time; findings which correspond to the endogenous tissue concentrations and metabolic path of these metabolites (Elverfors and Nissbrandt, 1991, 1992; Rutledge and Johanson, 1967; Westerink and Korf, 1976). The 3-MT fraction has negligible radioactivity, also in analogy with the very low endogenous concentrations in the brain (Westerink and Spaan, 1982; Wood and Altar, 1988). A major finding in ***Paper I*** is higher amount of radioactivity from the substantia nigra as compared to the striatum in the three front fractions (No. 1-3 in Fig. 2 in ***Paper I***), No. 2 and 3 containing inter alia NE and its metabolites. The opposite is, however, true for the DA fractions. The explanation for these differences are probably that NE-containing neurones are relatively more abundant in the substantia nigra than in the striatum, illustrated by a higher relative molar concentration of NE in the substantia nigra (≈ 30% of DA) in comparison to the striatum (≈ 5% of DA). Due to this difference, relatively more radioactive L-DOPA will be synthesized to NE in the substantia nigra and to DA in the striatum. In addition, there are lower ratios of DOPAC/DA and HVA/DA in the striatum verifying a slower DA turnover in this tissue (see below).

The fact that the total DPM of homogenate/g tissue was similar in the two nuclei is intriguing and signifies that the uptake/retention of radiolabelled L-DOPA-derived

products were similar in these regions, even though differences have been suggested (Cumming et al., 1994; Hartvig et al., 1992; Lindner et al., 1995). Intuitively, striatum should show higher radioactivity per g weight than the substantia nigra, due to its much higher DA concentration (~ 10 times higher) (Elverfors and Nissbrandt, 1992; Nissbrandt et al., 1985) which reflects DA synthesis, storage and release capacity. L-DOPA uptake in NE (see above) or 5-HT neurones (both being more abundant in the substantia nigra) does not provide sufficient capacity to explain the similarity in radioactive retention in the two brain parts. A more rapid fractional synthesis rate of DA in the substantia nigra compared to the striatum (Nissbrandt and Carlsson, 1987; Nissbrandt et al., 1985), founded on a relatively more rapid conversion of L-DOPA to DA and its metabolites could be an additional cause of this finding.

It should also be noted that L-DOPA-derived radioactivity tightly bound to tissue and therefore not present in the eluate following centrifugation, was not analysed. Theoretically, differences in the amount of such radioactivity between the substantia nigra and the striatum could account for the unexpected similarity between the brain parts. Such retention may be unspecific, i.e. consisting of radioactivity not derived from DA/NE-synthesis/metabolism and could in theory be examined by analysing brain structures devoid of DOPA decarboxylase. It is, however, not easy to find a suitable brain region to study for this purpose because monoaminergic neurones are widely distributed in the brain.

In short, the findings indicate that the method is suitable for studying the metabolism of L-DOPA and DA. Bearing in mind the mentioned aim of the study, i.e. searching for novel DA metabolites, it was important to gather fractions from the whole chromatogram, even in the absence of an apparent peak or known metabolite. Indeed, radioactivity was also seen in such fractions. Because the first 0-2 min fraction contained considerably higher amount of radioactivity than the estimated limit of impurities, it is likely that this fraction may represent *in vivo* generated products of L-DOPA. Further separation and analysis of this fraction will be an interesting future project. Furthermore, the other fractions not containing known metabolites should not be disregarded as sources for further investigation since some of these fractions consisted of as much or even more tritium than for example the HVA fractions. It is also interesting that radioactivity in fraction No. 2 became relatively higher in both nuclei following PEITC administration (Fig. 3 in ***Paper I***). In view of the short time elapsed after PEITC injection, the increased radioactivity could not be due to induction of the enzymes producing DOMA and DOPEG (see Fig. 2). An inhibition of COMT is neither probable because the radioactivity is not altered in fraction No. 3, containing VMA, nor in the HVA fraction (mean ± SEM value for HVA in substantia nigra: control = 0.021 ± 0.005, + PEITC = 0.033 ± 0.008; in the striatum: control = 0.032 ± 0.002, + PEITC = 0.037 ± 0.003; previously unpublished findings), both being metabolites produced by COMT.

Therefore, it is more likely that another L-DOPA derived molecule, which synthesis or degradation is affected by PEITC, is present in fraction No. 2. PEITC treatment did not however alter total tissue radioactivity indicating that uptake/retention of L-DOPA is not altered by the activity of CYP2E1 in either nuclei. In interpreting the present results, it is with vigilance that we propose the involvement of this enzyme in L-DOPA/DA metabolism. Better analysis of the front fractions is needed, such as separation of each NE metabolite. In the case of suspected novel metabolites complementary methods is necessary for identification.

Extracellular dopamine

The experimental approach in ***Paper II*** was undertaken in order to elucidate mechanisms responsible for the previously reported increase in nigral extracellular DA induced by three different CYP2E1 inhibitors. Animals receiving a pre-treatment of the DA uptake inhibitor GBR 12909 subsequent to PEITC administration, showed the same relative level of increase in extracellular DA concentration as those not pre-treated with GBR 12909. This finding points to an additive effect of these two drugs, implying that the effect of PEITC on extracellular DA is not via inhibition or reversal of the DA transporter. Likewise, the outcome of co-administration of PEITC and GBL was an apparent increase in extracellular DA concentration, indicating absence of interference of PEITC on the firing rate. Notably, treatment with GBL only caused a modest decrease in nigral extracellular DA in contrast to the effect seen in the striatum, illustrating a possible lack of total dependency of DA release on neuronal firing of dopaminergic substantia nigra neurones.

The abrupt decrease in extracellular nigral DA concentration caused by TTX perfusion confirms that nigral DA release relies on locally functional voltage dependent sodium channels, in line with previous reports (Santiago and Westerink, 1992). An added effect of TTX could be local blockade of the excitatory input (action potential) to the dopaminergic neurones, causing a somewhat larger decline in DA release as compared to the relatively moderate effect of GBL administration. The increase in DA release induced by PEITC treatment alone was not observed during TTX perfusion indicating that the effect of PEITC is on the physiological releasable pool of DA and points against the possibility of unspecific toxic aftermath by PEITC. This conclusion was further validated during treatment with reserpine, where the decline in extracellular DA concentration in the substantia nigra caused by reserpine was maintained also after PEITC delivery.

The TTX and reserpine data could indicate that the increasing effect of PEITC on nigral extracellular DA is absent due to a very low extracellular DA pool. Therefore, it would be tempting to conclude that the effect of PEITC is executed on released DA and postsynaptic to the dopaminergic neurones. This must be considered as one possibility.

For example, CYP2E1 has been found in glia cells, although not in the substantia nigra (Watts, et al., 1998). However, if the interaction by PEITC is postsynaptic, the effect of PEITC following GBL pre-treatment would not be so large, rather a doubling of the DA concentration following GBL administration would be expected. Another possibility is that the effect of PEITC is presynaptic and that PEITC increases the amount of DA that is available for release, implying that what we observe following PEITC is an increased release of DA. A morphological prerequisite for such an effect ought to be that the target for PEITC, presumably CYP2E1, is located near or in the same compartment as stored DA in the SN. Interestingly, morphological data support the idea of a co-localisation of DA and CYP2E1. The intracellular distribution of CYP2E1 in brain tissue is still a matter under study but could encompass the same pattern of distribution as in hepatocytes, i.e. *inter alia* bound to the ER (see "Cytochrome P450 2E1" in "Introduction" above). The main storage site for DA in dendrites is proposed to be situated in the ER where association with tyrosine hydroxylase has been found. Thus, both previous morphological data and the pharmacological findings in the present study support the presence of DA and CYP2E1 in the same vicinity in the ER.

The fact that an increase in DA radioactivity was not observed during the investigations conducted in ***Paper I*** following PEITC is likely due to different experimental procedures. It is important to point out that supernatant of homogenized tissue contains both intra- and extracellular DA and that the overwhelming part of DA in supernatant originates from the intracellular pool of DA. Therefore, it is likely that the increase in extracellular DA caused by PEITC is masked by the large unaffected amount of intracellular DA.

PEITC as a CYP2E1 inhibitor

There are at present no selective CYP2E1 inhibitors available. Available CYP2E1 inhibitors, apart from interfering more or less with other CYP isoenzymes, also affect other enzymes and even other targets for drugs. In the study by Nissbrandt (Nissbrandt et al., 2001), three different inhibitors of CYP2E1 were employed. PEITC demonstrated the most robust effect on DA release in the substantia nigra and therefore the drug at the exploited dose became the drug of choice in our investigations. To investigate dose-response relationship for PEITC was beyond the scope of this project. Although the inhibitory effect of PEITC *in vivo* on the activity of CYP2E1 in the brain has not yet been investigated, there are reasons to believe that the dose used in the present study would induce a considerable decrease of the activity of the enzyme in the brain. Intragastric administration of PEITC to rat, in doses comparable to the dose used in the present study, have been shown to induce an inhibition of liver CYP2E1 activity by at least 70-80 % (Lindros et al., 1995; Reicks and Crankshaw, 1996). Furthermore, the drug will most likely pass the blood-brain barrier as it is highly lipophilic.

One important matter throughout both investigations is the selectivity of PEITC as an inhibitor of CYP2E1. This drug is a competitive inhibitor of the enzyme exhibiting a relatively low K_i value for the enzyme (Ishizaki et al., 1990; Jiao et al., 1996; Leclercq et al., 1998; Lindros et al., 1995). However, mechanism-based invactivation of the enzyme by PEITC has also been reported, leading to destruction of the apoprotein (Moreno et al., 2001; Nakajima et al., 2001). This drug has also been mentioned as an inhibitor of aldehyde dehydrogenase (Koivusalo and Lindros, 1997). The latter enzyme mediates the last step in the synthesis of HVA and DOPAC (Fig.2). Our previous (Nissbrandt et al., 2001) and current findings show no effect on the concentrations of these metabolites following PEITC treatment which argues against such an effect of the drug at the present dose.

Some studies have claimed the inhibition of CYP1A1, CYP1A2, CYP3A4, CYP2B1, CYP2B6 and CYP2D6 by this substance (Conaway et al., 1996; Morris et al., 2004; Nakajima et al., 2001; Smith et al., 1996; Thapliyal and Maru, 2001). Yet, absence of immunoreactivity in nigral cells for CYP1A1, CYP1A2 and CYP2B1 in rat brain (Riedl et al., 1996; Watts et al., 1996), and also lack of protein expression for CYP2D6 in a post-mortem study of human brain (Siegle et al., 2001) have been reported. As for CYP2B6, only an un-inducible level of this enzyme has been revealed in the substantia nigra (as compared to 12 other brain regions) of the African green monkey (Lee et al., 2006). Expression of CYP3A4 has not yet been studied in the latter brain part. Altogether, these findings argue against an effect of this drug through the action of these enzymes in this brain region.

A hypothetical mechanism of action of PEITC would be an inhibitory effect on DBH, the enzyme converting DA to NE. Although, there are no investigations reporting such an effect, an inhibition of DBH would theoretically induce an increase of DA, released from NE containing neurones, and would explain the difference of effect of the drug in the striatum and the substantia nigra, since NE content in the former brain part is relatively much sparse than in the substantia nigra. Such an effect can however be ruled out since PEITC has no effect in the adjacent DA cell body region the ventral tegmental area (VTA) (see Fig. 9, unpublished findings), where the ratio of extracellular NE/DA is 2 - 6 (Chen and Reith, 1995; Gronier et al., 2000), whereas in the substantia nigra the ratio is much less, 0,3 - 0,5 (data from our laboratory). Furthermore, the amount of radioactivity in fractions Nos. 2-3, containing NE and its metabolites were actually unaffected or increased following PEITC treatment (***Paper I***).

In recent years, PEITC and other thiocyanates have been shown to have anti-cancer properties that include the ability to trigger apoptosis (Ferguson and Philpott, 2007; Wu and Hua, 2007). There are many theories behind this effect, one is that PEITC binds to GSH and thereby decreases the cell's defense against ROS (Kolm et al., 1995; Zhang et

al., 1995). Since ROS production is dramatically increased in cancer cells, these cells are predominantly vulnerable to decreased GSH concentrations and subsequent increased oxidative stress (Franco et al., 2007). Data supporting this mechanism originates mostly from *in vitro* studies on cancer cell lines (Trachootham et al., 2006; Wu et al., 2005; Yu et al., 1998; Zhang et al., 2003) and *in vivo* studies are scares (Nishikawa et al., 2004), showing contradictory results (Okulicz et al., 2005). The effect of this drug on GSH levels in normal cells is not yet clear and needs to be evaluated, especially in brain regions prone to oxidative stress. Nevertheless, it is not far fetched to speculate that a possible GSH reduction caused by a single administration of this drug (as in the present performed experiments) would not have a major consequence on cell survival.

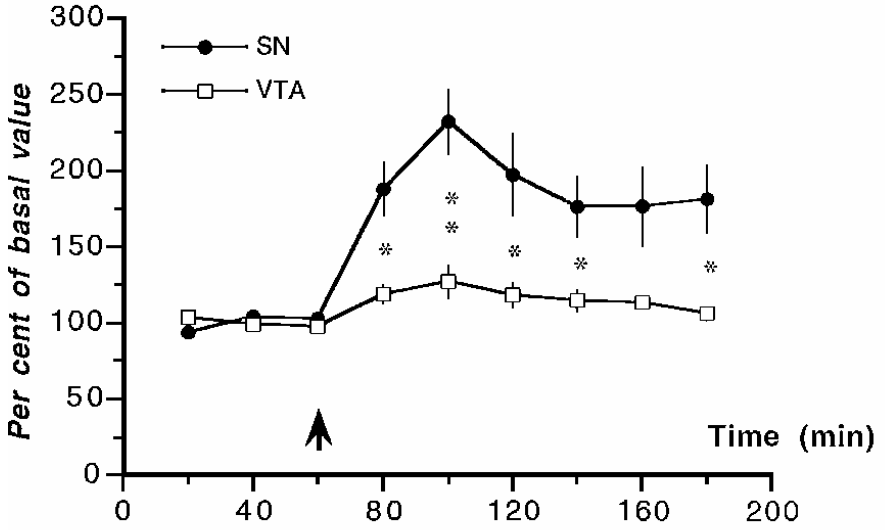

Figure 9. *The effect of phenylethyl isothiocyanate (100 mg/Kg i.p.) on the concentration of dopamine (DA) in dialysate from the substantia nigra (SN) and the ventral tegmental area (VTA). Phenylethyl isothiocyanate was given at arrow. Shown are mean concentrations ± SEM. n = 3-5. The mean concentration of three consecutive 20 min samples before drug treatment was considered as basal value and set to 100%. Basal concentrations of DA (fmol/uL) at 60 min were in the SN = 0.345 ± 0.029 and VTA = 0.69 ± 0.12. Differences between the groups were statistically evaluated by Student's t-test. * P < 0.05, ** P < 0.01.*

Although, there is no proof that the effects of PEITC are mediated by CYP2E1 inhibition, the drug clearly inhibits the enzyme and the overall pattern of effects point to an important contribution by CYP2E1 inhibition.

Localised effects of PEITC

CYP2E1 has been reported to be located in the substantia nigra, but also the olfactory lobes, cerebellum and hippocampus, prefrontal cortex, the accumbens, the striatum and the dopaminergic cell body region, the tegmental area (VTA) (Joshi and Tyndale, 2006b; Sanchez-Catalan et al., 2008; Yadav et al., 2006). Although the enzyme is localised in the striatum, substantia nigra and the VTA, significant effects of PEITC inhibitors on extracellular DA has only been seen in the substantia nigra, but not in the striatum or the VTA (Nissbrandt et al., 2001 and Fig. 9 in this thesis). An explanation for this mismatch could be that the enzyme has different functions in different brain parts. This is not unlikely considering the enzyme's broad substrate specificity. Another, more plausible reason could be localisation of the enzyme in different cell populations in different brain parts. In the striatum CYP2E1 has been found in striatal blood vessels (Riedl et al., 1996), in astrocytes of hippocampus and cortex (Tindberg et al., 1996) and in dopaminergic neurones in the substantia nigra (Watts et al., 1998).

It is, however, difficult to explain the similar effect of PEITC in the striatum and the substantia nigra on the L-DOPA derived radioactivity as compared to the selective effect of the drug on extracellular DA, seen only in the substantia nigra but not in the striatum (Nissbrandt et al., 2001). One obvious reason could be that the two findings are due to separate and different effects of the drug, implying that there are no causal relationship between the two different effects. Another possibility is that the underlying mechanism responsible for the effects seen on radioactivity is coupled to an increase in extracellular DA but only in the substantia nigra, due to different regulation of DA release in the two brain parts. Different regulation of DA neurotransmission in the terminal region (the striatum) and the cell body/dendritic region (substantia nigra) is not unlikely. Some differences have in fact been reported regarding DA storage and regulation of DA synthesis and release (see Introduction), although at present, the knowledge is insufficient as a basis for speculation regarding the different effects in the two brain parts.

ROS following isoflurane anaesthesia

Isoflurane anaesthesia significantly increased the formation of 3, 4-HB as compared to ketamine-xylazine anaesthesia, indicating an increased production of $^{\bullet}OH$. Isoflurane is considered to be mainly metabolized by CYP2E1 in both human and rat (Bradshaw and Ivanetich, 1984; Kharasch et al., 1999; Kharasch and Thummel, 1993) and metabolites of the compound have been measured in the urine and plasma, four days after administration, with the highest concentration after 2-3 days (Kharasch et al., 1999). One interpretation is that isoflurane anaesthesia induced the increase of ROS by CYP2E1 metabolism of the anaesthetic in the substantia nigra, as CYP2E1 is known to produce

free radical species during its catalytical cycle by means of an uncoupled reaction (Karuzina and Archakov, 1994a, b; Ronis et al., 1996; Staudt et al., 1974).

The interpretation of the finding is, however, complicated by other reported effects of isoflurane related to ROS production but not necessarily to CYP2E1. The anaesthetic has been reported to induce DNA damage and lipid peroxidation directly after administration, reinforced by simultaneous ethanol administration, indicative of oxidative stress, in brain tissue but also in other organs (Kim et al., 2006). However, an in vitro study on primary cortical neurons failed to show any ROS production as measured immediately after isoflurane discontinuation (Wang et al., 2008). Isoflurane has also been suggested to abruptly increase ROS production in the heart by stimulating the superoxide anion production in mitochondria (Tanaka et al., 2002). In addition, liver metabolites of isoflurane have been claimed to be reactive halides capable of triflouroacetylating proteins (Kharasch et al., 1999). Thus, a toxic effect in the CNS of such metabolites is theoretically possible. Finally, isoflurane has been shown to increase extracellular DA in the striatum (Adachi et al., 2005; Adachi et al., 2008), suggested to be caused by increased firing rate of dopaminergic neurones, in turn due to an interaction of the drug with A-type and delayed rectifier K channels (Ishiwa et al., 2008). However, the DA concentration returned to baseline 1 – 2 h after isoflurane administration making it less likely that the effect observed in the present study is caused by extracellular DA release. Furthermore, the effect of isoflurane on nigral DA has not been investigated.

Another possibility is that the ROS production measured after isoflurane anaesthesia is the normal production in brain tissue following probe implantation and that ketamine/xylazine inhibits the production or binds to ROS. Ketamine and its metabolites have been detected in monkeys long after its administration (Negrusz et al., 2005). Ketamine is an antagonist of NMDA glutamate receptors and ought to diminish glutamate toxicity which theoretically could be of relevance in the vicinity of an implanted probe. This drug has also been implicated to be involved in the formation of carbon-centered radicals by means of scavenging $^{\bullet}OH$ (Reinke et al., 1998), but this effect is weak and no long lasting ability of this drug regarding ROS production has been reported. The effect of ketamine on ROS in brain has to our knowledge, never been measured; although an investigation on ROS production in human leukocytes failed to show any effect of the drug (Nishina et al., 1998). However, some antioxidant effect in direct connection to ketamine administration has been reported in another study (Kang et al., 1998).

Considering the findings in the present study and in view of the theories involved in initiation and progression of PD (see above) together with the occurrence of CYP2E1 in the substantia nigra, it is alluring to propose that an increase in production of ROS due to CYP2E1 activity could be one underlying factor in this disease.

Genetic studies

CYP2E1 gene polymorphisms

The results obtained in ***Paper III***, following the comparative analysis of CYP2E1 gene polymorphism in PD patients and controls indicated a possible association between a C/G polymorphism at intron 7 and the disease, showing an increased frequency of the C allele in PD patients. This association was more robust when comparing only PD patients without PD relatives to controls. The latter finding was surprising because intuitively, presence of PD relatives would indicate a more significant genetic influence on the pathophysiology than in PD patients without known relatives, although presence of PD relatives could instead indicate exposure to similar environmental risk factors for PD. It is possible that the result in the present study points to a gene-environment interaction. It would have been interesting to compare polymorphism frequencies between a PD population and a control population exposed to similar environmental factors known to increase the risk for PD. However, because environmental risk factors for sporadic PD are mainly unknown such a comparison is presently impossible.

An earlier study investigating the functional role of this and two other polymorphisms of the CYP2E1 gene for chlorzoxazone hydroxylation capacity in human liver cells demonstrated high inter-individual diversity in enzyme activity (Carriere et al., 1996), but no correlation to the polymorphic forms. This finding weakens the possibility of this SNP to be directly related to catalytic capacity. Also, being an intronic SNP, the likelihood of an influence on catalytic capacity or substrate specificity is rather small.

However, variations in intronic SNPs could have other effects such as influencing splicing or regulatory processes by affecting the binding of transcriptions factors to the gene. By influencing the regulatory process of the gene, these SNPs could in combination with exogenous influences affect the enzyme's production of ROS or its capability to detoxify potential neurotoxins.

As a tSNP, this polymorphism is representative of many other variants which could be regulatory in the function of the enzyme. Also, the investigated SNP may be in linkage disequilibrium with functional variants. In a recent investigation (Singh et al., 2008), examining the CYP2E1*5B (Rsa I), CYP2E1*6 (Dra I) and CYP2D6*4 (1934G/A) polymorphism, an increased risk of association to PD through the gene-gene interaction of CYP2D6*4 and CYP2E1*5 was proposed. The statistic power in the mentioned study was however weak due to very few number of patients exhibiting this association. Nevertheless, the theory of linkage of SNPs, either within or in diverse genes, as a probable risk factor in developing PD is not far fetched. Additional analysis including

other aspects such as familial genetics, smoking and dietary habits is required in order to determine the exact role of polymorphisms of CYP2E1 in relation to PD.

Spliced variants in human brain

Investigations conducted in ***Paper IV*** on the expression of CYP2E1 showed that the enzyme is present in putamen and caudate nucleus of five individuals, exhibiting two alternative spliced forms. The isoform displaying 1068 bp, denoted GenBank mRNA sequence AJ853939, has previously only been observed in human lung cancer cell lines, hepatocyte cell lines and blood leukocytes (Bauer et al., 2005). Different spliced forms of enzymes belonging to other CYP450 enzyme subfamilies (e.g. CYP2D) have been identified in various tissues including the brain (Turman et al., 2006), therefore the presence of alternatively spliced forms of CYP2E1 in this organ is not implausible. Knowing the importance of alternative spliced variant of mRNAs, which extensively contribute to the excistence of modified protein products (Modrek and Lee, 2002), diverse spliced forms of CYP2E1 could indicate differential activity, substrate affinity or ROS generating capability for these isoforms, affecting the metabolism of both endo- and exogenous substances and possibly their ability to induce oxidative stress. We demonstrate that CYP2E1 exhibits both intra- and inter-brain nuclei transcriptional variations in humans. Such discrepancies could signify a basis of susceptibility to PD and clarifying the functional difference of the alternatively spliced forms of this enzyme would be of interest. It is noteworthy to mention that the predicted protein quality for the spliced form AJ853939 is recorded as “very good” according to AceView, whereas in the study performed by Bauer (Bauer et al, 2005), this transcript is characterised as truncated and not functional. Further studies are necessary for revealing the presence of CYP2E1 isoforms and their functional importance in other parts of the brain that are of pathophysiological importance for PD, e.g. substantia nigra.

Conclusions, reflections and future research

CYP450 enzymes are the main proteins responsible for the metabolism of xenobiotics and many hydrophobic compounds in the brain may be transformed to hydrophilic metabolites by these enzymes, either detoxified to less harmful compounds or bio-activated to toxic metabolites. Some of these enzymes, inter alia CYP2E1, have also been associated with production of ROS. The relatively low capacity of the defence against ROS in the brain in combination with its highly unsaturated lipid content makes the CNS vulnerable for oxidative stress. Furthermore, regeneration and repair of damaged tissue in the brain is limited. In the present investigations, we have studied CYP2E1 based on a possible connection to dopaminergic neurotransmission and PD pathophysiology.

The occurrence of CYP2E1 in the brain and induction of the enzyme by different substances are indications of a physiological role. The enzyme is localised in diverse parts and cell populations and could due to its broad substrate specificity have different functions in different brain parts. Even thought the concentration of CYP2E1 in the brain is much lower than in the liver, the enzyme could be important for detoxification and metabolism of endo- and exogenous substances in specific regions of the CNS.

Our results indicate that CYP2E1, directly or indirectly, influences DA neurotransmission in the substantia nigra, via affecting DA metabolism or release, and that it may produce ROS. We are well aware that the association of some of the findings obtained in the present animal studies to CYP2E1 is indirect and founded on the effects of drugs that also have other effects than influencing or being metabolised by CYP2E1. Therefore the interpretations must be drawn with caution. The scope of the present studies was to highlight the possible involvement of CYP2E1 in physiological and pathophysiological processes in the substantia nigra and stimulate further studies utilizing other techniques. More conclusive studies are required to strengthen the results obtained in the present thesis. The lack of specific pharmacolocical tools is, however, a drawback for *in vivo* research on CYP2E1. Utilizing, CYP2E1 knock-out mice as controls in future experiments would be of great value, although performing microdialysis study measuring DA or ROS in the substantia nigra of these animals would be a difficult task. Other approaches would be to utilise several different compounds known to interfere with CYP2E1; drugs with different molecular structures and therefore less probable to affect unwanted pharmacological targets. A microdialysis study, measuring the release of DA following induction of CYP2E1 would also be a suitable complementary investigation for better revealing this enzyme's influence on DA release. In addition, analysis of ROS production under the effect of inhibition or induction of CYP2E1 is necessary in order to obtain a more definite conclusion as to the role of this enzyme in oxidative stress.

In addition, our examinations point to a possible association of a polymorphic form of CYP2E1 and PD. Observations have also been made regarding tissue specific spliced variants of this enzyme in brain regions of interest for PD. Considering the existing presumptions on the pathophysiology of PD, it is noteworthy to point out that altered characteristics of this enzyme caused either by gene polymorphism, alternative splicing or substrate-caused alternations in its activity, can be susceptibility factors for this disease, reflecting gene-environmental interactions. For example, it is well established that smoking is negatively associated with PD, and several substances in cigarette smoke interact with CYP2E1, such as nicotine and carbon monoxide. An improved case-control founded trial considering also environmental history and parental genotypes are required to shed more light upon gene-environmental interactions regarding CYP2E1. Performance of such a trial is not without complexity as a common ecological cause of

this disease is not known and parental genotypes are often not available due to late onset and hence diagnosis of this disease. Nevertheless, CYP2E1 related factors such as alcohol, nicotine, dietary and xenobiotic intake habits can be categorized and included in a study to better evaluate the relevance of this enzyme in the development of PD.

In summary, we have developed a new method for studying the metabolism of catacholamines. The results also support the notion of a physiological role for CYP2E1 in the CNS, although at present it is premature to construct a model for its participation in dopaminergic neurotransmission and PD pathophysiology.

ACKNOWLEDGEMENTS

I would like to thank the following people:

My supervisor, Professor ***Hans Nissbrandt***, for giving me the opportunity to search for answers, guiding me in the path of science and for always explaining the most complicated matters of pharmacology on a "yellow post-it"(!!), trying to make them easier to understand. I hope to have captured and learned some small part of your immense knowledge in the field. I am deeply grateful.

Lars Westberg, for introducing me to the field of genetics, fruitful discussions and help with experiments. I appreciate it greatly.

Jonas Melke, for patients in helping me grasp and understand the fundamentals of genetics and for invaluable suggestions with the last two papers.

Lucia Gaete, for all her help in the lab (and always with a smile!), especially concerning my first paper. But most of all for being a good friend who always had time to have lunch with me when I needed to talk.

Anna Zettergren, for advice and help in understanding genetics, and of course friendship and pleasant conversations about every day life and parenthood.

Daniel Andersson, for helping me every time my computer refused to obey! And also help with the second paper.

Gunnar Tobin, for having time to answer questions about anything and everything at all times!

Annalena Carlred, for help with all the paperwork, for nice chats and always having time to listen and give support.

Inger Oscarsson and ***Gunilla Bourghardt,*** for help during genetic experiments and solving my problems at the lab, and mostly for enjoyable talks during lunch hours.

Britt-Marie Benbow, thank you for all your help with preparations of my dissertation and also during the past few years.

…and of course…

My parents, for giving me the courage and confidence to make this happen; my father for telling me to "think wisely" before making decisions and my mother for saying "don't think too much, it always works out in the end anyway"!

My sister Haleh and her family, for forgiving me for all the late postcards because of my work during the last year, and also for being the best sister and family one could wish for.

Gittan and Bert, for all the support they have given us during the past years.

♡ Last but not least, I want to thank my ever so understanding and patient husband, ***Marcus***, for love, encouragement, and for believing in me every step of the way. What would I have done without you! And of course … ***Melina***, for her unconditional love, and a smile which gives me energy and makes me the happiest person on earth.

References

Abdel-Razzak Z, Garlatti M, Aggerbeck M, Barouki R. 2004. Determination of interleukin-4-responsive region in the human cytochrome P450 2E1 gene promoter. Biochem Pharmacol 68(7):1371-1381.

Abdel-Razzak Z, Loyer P, Fautrel A, Gautier JC, Corcos L, Turlin B, Beaune P, Guillouzo A. 1993. Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. Mol Pharmacol 44(4):707-715.

Adachi YU, Yamada S, Satomoto M, Higuchi H, Watanabe K, Kazama T. 2005. Isoflurane anesthesia induces biphasic effect on dopamine release in the rat striatum. Brain Res Bull 67(3):176-181.

Adachi YU, Yamada S, Satomoto M, Higuchi H, Watanabe K, Kazama T, Mimuro S, Sato S. 2008. Isoflurane anesthesia inhibits clozapine- and risperidone-induced dopamine release and anesthesia-induced changes in dopamine metabolism was modified by fluoxetine in the rat striatum: an in vivo microdialysis study. Neurochem Int 52(3):384-391.

Aguiar M, Masse R, Gibbs BF. 2005. Regulation of cytochrome P450 by posttranslational modification. Drug Metab Rev 37(2):379-404.

Anandatheerthavarada HK, Boyd MR, Ravindranath V. 1992. Characterization of a phenobarbital-inducible cytochrome P-450, NADPH-cytochrome P-450 reductase and reconstituted cytochrome P-450 mono-oxygenase system from rat brain. Evidence for constitutive presence in rat and human brain. Biochem J 288 (Pt 2):483-488.

Anandatheerthavarada HK, Shankar SK, Bhamre S, Boyd MR, Song BJ, Ravindranath V. 1993a. Induction of brain cytochrome P-450IIE1 by chronic ethanol treatment. Brain Res 601(1-2):279-285.

Anandatheerthavarada HK, Williams JF, Wecker L. 1993b. Differential effect of chronic nicotine administration on brain cytochrome P4501A1/2 and P4502E1. Biochem Biophys Res Commun 194(1):312-318.

Antunes F, Nunes C, Laranjinha J, Cadenas E. 2005. Redox interactions of nitric oxide with dopamine and its derivatives. Toxicology 208(2):207-212.

Aoyama T, Nagata K, Yamazoe Y, Kato R, Matsunaga E, Gelboin HV, Gonzalez FJ. 1990. Cytochrome b5 potentiation of cytochrome P-450 catalytic activity demonstrated by a vaccinia virus-mediated in situ reconstitution system. Proc Natl Acad Sci U S A 87(14):5425-5429.

Bandmann O, Vaughan J, Holmans P, Marsden CD, Wood NW. 1997. Association of slow acetylator genotype for N-acetyltransferase 2 with familial Parkinson's disease. Lancet 350(9085):1136-1139.

Barbeau A, Cloutier T, Roy M, Plasse L, Paris S, Poirier J. 1985. Ecogenetics of Parkinson's disease: 4-hydroxylation of debrisoquine. Lancet 2(8466):1213-1216.

Barbeau A, Murphy GF, Sourkes TL. 1961. Excretion of dopamine in diseases of basal ganglia. Science 133:1706-1707.

Barbeau A, Murphy CF, Sourkes TL. 1962. Les catecolamines dans la maladie de Parkinson. In: Ajuriaguerra J, editor. Monoamines et systeme nerveux centrale. Paris: George, Geneve and Masso. p. 247-262.

Bar-Nun S, Kreibich G, Adesnik M, Alterman L, Negishi M, Sabatini DD. 1980. Synthesis and insertion of cytochrome P-450 into endoplasmic reticulum membranes. Proc Natl Acad Sci U S A 77(2):965-969.

Bartholini G, Pletscher A. 1969. Effect of various decarboxylase inhibitors on the cerebral metabolism of dihydroxyphenylalanine. J Pharm Pharmacol 21(5):323-324.

Bauer M, Herbarth O, Aust G, Graebsch C. 2005. Molecular cloning and expression of novel alternatively spliced cytochrome P450 2E1 mRNAs in humans. Mol Cell Biochem 280(1-2):201-207.

Baumane L, Dzintare M, Zvejniece L, Meirena D, Lauberte L, Sile V, Kalvinsh I, Sjakste N. 2002. Increased synthesis of nitric oxide in rat brain cortex due to halogenated volatile anesthetics confirmed by EPR spectroscopy. Acta Anaesthesiol Scand 46(4):378-383.

Benveniste H. 1989. Brain microdialysis. J Neurochem 52(6):1667-1679.

Benveniste H, Huttemeier PC. 1990. Microdialysis--theory and application. Prog Neurobiol 35(3):195-215.

Bergquist F, Jonason J, Pileblad E, Nissbrandt H. 1998. Effects of local administration of L-, N-, and P/Q-type calcium channel blockers on spontaneous dopamine release in the striatum and the substantia nigra: a microdialysis study in rat. J Neurochem 70(4):1532-1540.

Bertler A, Rosengren E. 1959. Occurrence and distribution of catechol amines in brain. Acta Physiol Scand 47:350-361.

Bhamre S, Anandatheerthavarada HK, Shankar SK, Ravindranath V. 1992. Microsomal cytochrome P450 in human brain regions. Biochem Pharmacol 44(6):1223-1225.

Bialecka M, Klodowska-Duda G, Honczarenko K, Gawronska-Szklarz B, Opala G, Safranow K, Drozdzik M. 2007. Polymorphisms of catechol-0-methyltransferase (COMT), monoamine oxidase B (MAOB), N-acetyltransferase 2 (NAT2) and cytochrome P450 2D6 (CYP2D6) gene in patients with early onset of Parkinson's disease. Parkinsonism Relat Disord 13(4):224-229.

Birkmayer W, Hornykiewicz O. 1962. [The L-dihydroxyphenylalanine (L-DOPA) effect in Parkinson's syndrome in man: On the pathogenesis and treatment of Parkinson akinesis.]. Arch Psychiatr Nervenkr Z Gesamte Neurol Psychiatr 203:560-574.

Blaschko H. 1939. The specific action of L-DOPA decarboxylase. J Physiol 96:50P.

Bonifati V, Rizzu P, Squitieri F, Krieger E, Vanacore N, van Swieten JC, Brice A, van Duijn CM, Oostra B, Meco G, Heutink P. 2003. DJ-1(PARK7), a novel gene for autosomal recessive, early onset parkinsonism. Neurol Sci 24(3):159-160.

Bowsher RR, Henry DP. 1985. Aromatic L-amino acid decarboxylase. Biochemistry and functional significance. In: Boulton AA, Baker GB, Yu PH, editors. Neuromethods, Series 1: Neruochemistry. New Jersey: Humana Press, Clifton. p. 33-78.

Bradshaw JJ, Ivanetich KM. 1984. Isoflurane: a comparison of its metabolism by human and rat hepatic cytochrome P-450. Anesth Analg 63(9):805-813.

Brady JF, Ishizaki H, Fukuto JM, Lin MC, Fadel A, Gapac JM, Yang CS. 1991a. Inhibition of cytochrome P-450 2E1 by diallyl sulfide and its metabolites. Chem Res Toxicol 4(6):642-647.

Brady JF, Xiao F, Wang MH, Li Y, Ning SM, Gapac JM, Yang CS. 1991b. Effects of disulfiram on hepatic P450IIE1, other microsomal enzymes, and hepatotoxicity in rats. Toxicol Appl Pharmacol 108(2):366-373.

Bras J, Paisan-Ruiz C, Guerreiro R, Ribeiro MH, Morgadinho A, Januario C, Sidransky E, Oliveira C, Singleton A. 2007. Complete screening for glucocerebrosidase mutations in Parkinson disease patients from Portugal. Neurobiol Aging.

Brissaud E. 1895. Lecons sur les Maladies Nerveuses. Paris: Masson et Cie.

Brown RC, Lockwood AH, Sonawane BR. 2005. Neurodegenerative diseases: an overview of environmental risk factors. Environ Health Perspect 113(9):1250-1256.

Brzezinski MR, Boutelet-Bochan H, Person RE, Fantel AG, Juchau MR. 1999. Catalytic activity and quantitation of cytochrome P-450 2E1 in prenatal human brain. J Pharmacol Exp Ther 289(3):1648-1653.

Buu NT, Duhaime J, Savard C, Truong L, Kuchel O. 1981. Presence of conjugated catecholamines in rat brain: a new method of analysis of catecholamine sulfates. J Neurochem 36(2):769-772.

Calon F, Rajput AH, Hornykiewicz O, Bedard PJ, Di Paolo T. 2003. Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. Neurobiol Dis 14(3):404-416.

Carlsson A. 1959. The occurrence, distribution and physiological role of catecholamines in the nervous system. Pharmacol Rev 11(2, Part 2):490-493.

Carlsson A. 2002. Treatment of Parkinson's with L-DOPA. The early discovery phase, and a comment on current problems. J Neural Transm 109(5-6):777-787.

Carlsson A, Lindqvist M. 1973. Effect of ethanol on the hydroxylation of tyrosine and tryptophan in rat brain in vivo. J Pharm Pharmacol 25(6):437-440.

Carlsson A, Lindqvist M, Magnusson T. 1957. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180(4596):1200.

Carlsson A, Lindqvist M, Magnusson T, Waldeck B. 1958. On the presence of 3-hydroxytyramine in brain. Science 127(3296):471.

Carlsson A, Waldeck B. 1964. A Method for the Fluorimetric Determination of 3-Methoxytyramine in Tissues and the Occurrence of This Amine in Brain. Scand J Clin Lab Invest 16:133-138.

Carriere V, Berthou F, Baird S, Belloc C, Beaune P, de Waziers I. 1996. Human cytochrome P450 2E1 (CYP2E1): from genotype to phenotype. Pharmacogenetics 6(3):203-211.

Cederbaum AI. 2006. CYP2E1--biochemical and toxicological aspects and role in alcohol-induced liver injury. Mt Sinai J Med 73(4):657-672.

Chan DK, Mellick GD, Buchanan DD, Hung WT, Ng PW, Woo J, Kay R. 2002. Lack of association between CYP1A1 polymorphism and Parkinson's disease in a Chinese population. J Neural Transm 109(1):35-39.

Chen NH, Reith ME. 1995. Monoamine interactions measured by microdialysis in the ventral tegmental area of rats treated systemically with (+/-)-8-hydroxy-2-(di-n-propylamino)tetralin. J Neurochem 64(4):1585-1597.

Chen R, Stenken JA. 2002. An in vitro hydroxyl radical generation assay for microdialysis sampling calibration. Anal Biochem 306(1):40-49.

Chien JY, Thummel KE, Slattery JT. 1997. Pharmacokinetic consequences of induction of CYP2E1 by ligand stabilization. Drug Metab Dispos 25(10):1165-1175.

Cohen G. 1994. Enzymatic/nonenzymatic sources of oxyradicals and regulation of antioxidant defenses. Ann N Y Acad Sci 738:8-14.

Cohn JA, Alvares AP, Kappas A. 1977. On the occurrence of cytochrome P-450 and aryl hydrocarbon hydroxylase activity in rat brain. J Exp Med 145(6):1607-1611.

Conaway CC, Jiao D, Chung FL. 1996. Inhibition of rat liver cytochrome P450 isozymes by isothiocyanates and their conjugates: a structure-activity relationship study. Carcinogenesis 17(11):2423-2427.

Coon MJ. 2005. Cytochrome P450: nature's most versatile biological catalyst. Annu Rev Pharmacol Toxicol 45:1-25.

Cooper JR, Bloom FE, Roth RH. 1996. The biochemical basis of neuropharmacology, 7th ed. New York: Oxford University Press. 231-251 p.

Correia MA, Sadeghi S, Mundo-Paredes E. 2005. Cytochrome P450 ubiquitination: branding for the proteolytic slaughter? Annu Rev Pharmacol Toxicol 45:439-464.

Cotzias GC, Van Woert MH, Schiffer LM. 1967. Aromatic amino acids and modification of parkinsonism. N Engl J Med 276(7):374-379.

Coutts RT, Urichuk LJ. 1999. Polymorphic cytochromes P450 and drugs used in psychiatry. Cell Mol Neurobiol 19(3):325-354.

Cumming P, Kuwabara H, Gjedde A. 1994. A kinetic analysis of 6-[18F]fluoro-L-dihydroxyphenylalanine metabolism in the rat. J Neurochem 63(5):1675-1682.

Dale RC, Church AJ, Surtees RA, Lees AJ, Adcock JE, Harding B, Neville BG, Giovannoni G. 2004. Encephalitis lethargica syndrome: 20 new cases and evidence of basal ganglia autoimmunity. Brain 127(Pt 1):21-33.

Daly AK, Cholerton S, Gregory W, Idle JR. 1993. Metabolic polymorphisms. Pharmacol Ther 57(2-3):129-160.

Damme B, Darmer D, Pankow D. 1996. Induction of hepatic cytochrome P4502E1 in rats by acetylsalicylic acid or sodium salicylate. Toxicology 106(1-3):99-103.

Daniel SE, Lees AJ. 1993. Parkinson's Disease Society Brain Bank, London: overview and research. J Neural Transm Suppl 39:165-172.

Danko IM, Chaschin NA. 2005. Association of CYP2E1 gene polymorphism with predisposition to cancer development. Exp Oncol 27(4):248-256.

de Carvalho Aguiar P, Lessa PS, Junior CG, Barsottini O, Felicio AC, Borges V, de Azevedo Silva SM, Saba RA, Ferraz HB, Moreira-Filho CA, Andrade LA. 2008. Genetic and environmental findings in early-onset Parkinson's disease Brazilian patients. Mov Disord.

De la Vega FM, Lazaruk KD, Rhodes MD, Wenz MH. 2005. Assessment of two flexible and compatible SNP genotyping platforms: TaqMan SNP Genotyping Assays and the SNPlex Genotyping System. Mutat Res 573(1-2):111-135.

De Palma G, Mozzoni P, Mutti A, Calzetti S, Negrotti A. 1998. Case-control study of interactions between genetic and environmental factors in Parkinson's disease. Lancet 352(9145):1986-1987.

de Rijk MC, Launer LJ, Berger K, Breteler MM, Dartigues JF, Baldereschi M, Fratiglioni L, Lobo A, Martinez-Lage J, Trenkwalder C, Hofman A. 2000. Prevalence of Parkinson's disease in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology 54(11 Suppl 5):S21-23.

de Waziers I, Cugnenc PH, Yang CS, Leroux JP, Beaune PH. 1990. Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. J Pharmacol Exp Ther 253(1):387-394.

Deng Y, Newman B, Dunne MP, Silburn PA, Mellick GD. 2004. Further evidence that interactions between CYP2D6 and pesticide exposure increase risk for Parkinson's disease. Ann Neurol 55(6):897.

Doder M, Rabiner EA, Turjanski N, Lees AJ, Brooks DJ. 2003. Tremor in Parkinson's disease and serotonergic dysfunction: an 11C-WAY 100635 PET study. Neurology 60(4):601-605.

Double KL, Gerlach M, Youdim MB, Riederer P. 2000. Impaired iron homeostasis in Parkinson's disease. J Neural Transm Suppl(60):37-58.

Eblan MJ, Nguyen J, Ziegler SG, Lwin A, Hanson M, Gallardo M, Weiser R, De Lucca M, Singleton A, Sidransky E. 2006. Glucocerebrosidase mutations are also found in subjects with early-onset parkinsonism from Venezuela. Mov Disord 21(2):282-283.

Ehringer H, Hornykiewicz O. 1960. [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system.]. Klin Wochenschr 38:1236-1239.

Ekstrom G, Ingelman-Sundberg M. 1984. Cytochrome P-450-dependent lipid peroxidation in reconstituted membrane vesicles. Biochem Pharmacol 33(15):2521-2523.

Ekstrom G, Ingelman-Sundberg M. 1989. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). Biochem Pharmacol 38(8):1313-1319.

Elbaz A, Levecque C, Clavel J, Vidal JS, Richard F, Amouyel P, Alperovitch A, Chartier-Harlin MC, Tzourio C. 2004. CYP2D6 polymorphism, pesticide exposure, and Parkinson's disease. Ann Neurol 55(3):430-434.

Elverfors A, Nissbrandt H. 1991. Reserpine-insensitive dopamine release in the substantia nigra? Brain Res 557(1-2):5-12.

Elverfors A, Nissbrandt H. 1992. Effects of d-amphetamine on dopaminergic neurotransmission; a comparison between the substantia nigra and the striatum. Neuropharmacology 31(7):661-670.

Elverfors A, Pileblad E, Lagerkvist S, Bergquist F, Jonason J, Nissbrandt H. 1997. 3-Methoxytyramine formation following monoamine oxidase inhibition is a poor index of dendritic dopamine release in the substantia nigra. J Neurochem 69(4):1684-1692.

Emery MG, Jubert C, Thummel KE, Kharasch ED. 1999. Duration of cytochrome P-450 2E1 (CYP2E1) inhibition and estimation of functional CYP2E1 enzyme half-life after single-dose disulfiram administration in humans. J Pharmacol Exp Ther 291(1):213-219.

Engberg G, Elverfors A, Jonason J, Nissbrandt H. 1997. Inhibition of dopamine re-uptake: significance for nigral dopamine neuron activity. Synapse 25(2):215-226.

Fagervall I, Ross SB. 1986. A and B forms of monoamine oxidase within the monoaminergic neurons of the rat brain. J Neurochem 47(2):569-576.

Fahn S, Sulzer D. 2004. Neurodegeneration and neuroprotection in Parkinson disease. NeuroRx 1(1):139-154.

Fakhrai-Rad H, Pourmand N, Ronaghi M. 2002. Pyrosequencing: an accurate detection platform for single nucleotide polymorphisms. Hum Mutat 19(5):479-485.

Farin FM, Omiecinski CJ. 1993. Regiospecific expression of cytochrome P-450s and microsomal epoxide hydrolase in human brain tissue. J Toxicol Environ Health 40(2-3):317-335.

Ferguson LR, Philpott M. 2007. Cancer prevention by dietary bioactive components that target the immune response. Curr Cancer Drug Targets 7(5):459-464.

Fernandez V, Massa L, Quinones L, Simon-Giavarotti KA, Giavarotti L, D'Almeida V, Azzalis LA, Junqueira VB, Videla LA. 2003. Effects of gamma-hexachlorocyclohexane and L-3,3',5-triiodothyronine on rat liver cytochrome P4502E1-dependent activity and content in relation to microsomal superoxide radical generation. Biol Res 36(3-4):359-365.

Ferrari MD, de Wolff FA, Vermey P, Veenema H, Buruma OJ. 1986. Hepatic cytochrome P450 malfunction and Parkinson's disease. Lancet 1(8476):324.

Ferrari MD, Peeters EA, Haan J, Roos RA, Vermey P, de Wolff FA, Buruma OJ. 1990. Cytochrome P450 and Parkinson's disease. Poor parahydroxylation of phenytoin. J Neurol Sci 96(2-3):153-157.

Fitzmaurice PS, Ang L, Guttman M, Rajput AH, Furukawa Y, Kish SJ. 2003. Nigral glutathione deficiency is not specific for idiopathic Parkinson's disease. Mov Disord 18(9):969-976.

Franco R, Schoneveld OJ, Pappa A, Panayiotidis MI. 2007. The central role of glutathione in the pathophysiology of human diseases. Arch Physiol Biochem 113(4-5):234-258.

Funk C. 1911. Synthesis of dl-3 : 4-Dihydroxyphenylalamine. J Chem Soc 99:554-557.

Gasser T. 2005. Genetics of Parkinson's disease. Curr Opin Neurol 18(4):363-369.

Gibson G, Skett P. 1994. Introduction to drug metabolism. London: Blackie Academic & Professional.

Gilham DE, Cairns W, Paine MJ, Modi S, Poulsom R, Roberts GC, Wolf CR. 1997. Metabolism of MPTP by cytochrome P4502D6 and the demonstration of 2D6 mRNA in human foetal and adult brain by in situ hybridization. Xenobiotica 27(1):111-125.

Gonzalez FJ. 1988. The molecular biology of cytochrome P450s. Pharmacol Rev 40(4):243-288.

Gonzalez FJ, Gelboin HV. 1990. Transcriptional and posttranscriptional regulation of CYP2E1, an N-nitrosodimethylamine demethylase. Princess Takamatsu Symp 21:157-164.

Gonzalez FJ. 2007. The 2006 Bernard B. Brodie Award Lecture. Cyp2e1. Drug Metab Dispos 35(1):1-8.

Gorell JM, Rybicki BA, Johnson CC, Peterson EL. 1999. Smoking and Parkinson's disease: a dose-response relationship. Neurology 52(1):115-119.

Gronier B, Perry KW, Rasmussen K. 2000. Activation of the mesocorticolimbic dopaminergic system by stimulation of muscarinic cholinergic receptors in the ventral tegmental area. Psychopharmacology (Berl) 147(4):347-355.

Groves PM, Linder JC. 1983. Dendro-dendritic synapses in substantia nigra: descriptions based on analysis of serial sections. Exp Brain Res 49(2):209-217.

Guggenheim M. 1913. Dioxyphenylalanin, eine neue Aminosäure aus Vicia faba. Hoppe-Seyler's Z. Physiol Chem 88:276-284.

Gudjonsson O, Sanz E, Alvan G, Aquilonius SM, Reviriego J. 1990. Poor hydroxylator phenotypes of debrisoquine and S-mephenytoin are not over-represented in a group of patients with Parkinson's disease. Br J Clin Pharmacol 30(2):301-302.

Guldberg HC, Marsden CA. 1975. Catechol-O-methyl transferase: pharmacological aspects and physiological role. Pharmacol Rev 27(2):135-206.

Hagell P, Cenci MA. 2005. Dyskinesias and dopamine cell replacement in Parkinson's disease: a clinical perspective. Brain Res Bull 68(1-2):4-15.

Hanioka N, Tanaka-Kagawa T, Miyata Y, Matsushima E, Makino Y, Ohno A, Yoda R, Jinno H, Ando M. 2003. Functional characterization of three human cytochrome p450 2E1 variants with amino acid substitutions. Xenobiotica 33(6):575-586.

Hansson T, Tindberg N, Ingelman-Sundberg M, Kohler C. 1990. Regional distribution of ethanol-inducible cytochrome P450 IIE1 in the rat central nervous system. Neuroscience 34(2):451-463.

Hartvig P, Lindner KJ, Tedroff J, Bjurling P, Hornfelt K, Langstrom B. 1992. Regional brain kinetics of 6-fluoro-(beta-11C)-L-dopa and (beta-11C)-L-dopa following COMT inhibition. A study in vivo using positron emission tomography. J Neural Transm Gen Sect 87(1):15-22.

Hattori T, McGeer PL, McGeer EG. 1979. Dendro axonic neurotransmission. II. Morphological sites for the synthesis, binding and release of neurotransmitters in dopaminergic dendrites in the substantia nigra and cholinergic dendrites in the neostriatum. Brain Res 170(1):71-83.

Hefti F, Lienhart R, Lichtensteiger W. 1976. Transmitter metabolism in substantia nigra after inhibition of dopaminergic neurones by butyrolactone. Nature 263(5575):341-343.

Heimer L, Alheid GF. 1985. Basal Ganglia. In: Paxinos, editor. The rat nervous system. New York: Academic Press.

Herraiz T, Guillen H, Aran VJ, Idle JR, Gonzalez FJ. 2006. Comparative aromatic hydroxylation and N-demethylation of MPTP neurotoxin and its analogs, N-methylated beta-carboline and isoquinoline alkaloids, by human cytochrome P450 2D6. Toxicol Appl Pharmacol 216(3):387-398.

Hiroi T, Imaoka S, Funae Y. 1998. Dopamine formation from tyramine by CYP2D6. Biochem Biophys Res Commun 249(3):838-843.

Ho SL, McCann KP, Bennett P, Kapadi AL, Waring RH, Ramsden DB, Williams AC. 1996. The molecular biology of xenobiotic enzymes and the predisposition to idiopathic Parkinson's disease. Adv Neurol 69:53-60.

Hoffman AF, Gerhardt GA. 1999. Differences in pharmacological properties of dopamine release between the substantia nigra and striatum: an in vivo electrochemical study. J Pharmacol Exp Ther 289(1):455-463.

Holtz P. 1939. Dopadecarboxylase. Naturwissenschaften 27:724-725.

Hong JY, Pan JM, Gonzalez FJ, Gelboin HV, Yang CS. 1987. The induction of a specific form of cytochrome P-450 (P-450j) by fasting. Biochem Biophys Res Commun 142(3):1077-1083.

Hornykiewicz O. 1986. A quarter century of brain dopamine research. In: Woodruff G, Poat JA, Roberts PJ, editor. Dopaminergic systems and their regulation. London: Macmillan. p. 3-18.

Hu Y, Oscarson M, Johansson I, Yue QY, Dahl ML, Tabone M, Arinco S, Albano E, Ingelman-Sundberg M. 1997. Genetic polymorphism of human CYP2E1: characterization of two variant alleles. Mol Pharmacol 51(3):370-376.

Iba MM, Fung J, Gonzalez FJ. 2000. Functional Cyp2e1 is required for substantial in vivo formation of 2,5-hexanedione from n-hexane in the mouse. Arch Toxicol 74(10):582-586.

Iba MM, Scholl H, Fung J, Thomas PE, Alam J. 1998. Induction of pulmonary CYP1A1 by nicotine. Xenobiotica 28(9):827-843.

Imperato A, Di Chiara G. 1984. Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: a new method for the study of the in vivo release of endogenous dopamine and metabolites. J Neurosci 4(4):966-977.

Ingelman-Sundberg M. 2004. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. Naunyn Schmiedebergs Arch Pharmacol 369(1):89-104.

Ingelman-Sundberg M, Johansson I. 1984. Electron flow and complex formation during cytochrome P-450-catalyzed hydroxylation reactions in reconstituted membrane vesicles. Acta Chem Scand B 38(10):845-851.

Ishiwa D, Nagata I, Ohtsuka T, Itoh H, Kamiya Y, Ogawa K, Sakai M, Sekino N, Yamada Y, Goto T, Andoh T. 2008. Differential effects of isoflurane on A-type and delayed rectifier K channels in rat substantia nigra. Eur J Pharmacol 580(1-2):122-129.

Ishizaki H, Brady JF, Ning SM, Yang CS. 1990. Effect of phenethyl isothiocyanate on microsomal N-nitrosodimethylamine metabolism and other monooxygenase activities. Xenobiotica 20(3):255-264.

Jahng JW, Houpt TA, Wessel TC, Chen K, Shih JC, Joh TH. 1997. Localization of monoamine oxidase A and B mRNA in the rat brain by in situ hybridization. Synapse 25(1):30-36.

Jankovic J. 2006. An update on the treatment of Parkinson's disease. Mt Sinai J Med 73(4):682-689.

Jenner P. 1991. Oxidative stress as a cause of Parkinson's disease. Acta Neurol Scand Suppl 136:6-15.

Jenner P. 1998. Oxidative mechanisms in nigral cell death in Parkinson's disease. Mov Disord 13 Suppl 1:24-34.

Jenner P. 2003. Oxidative stress in Parkinson's disease. Ann Neurol 53 Suppl 3:S26-36; discussion S36-28.

Jenner P, Olanow CW. 1998. Understanding cell death in Parkinson's disease. Ann Neurol 44(3 Suppl 1):S72-84.

Jiao D, Conaway CC, Wang MH, Yang CS, Koehl W, Chung FL. 1996. Inhibition of N-nitrosodimethylamine demethylase in rat and human liver microsomes by isothiocyanates and their glutathione, L-cysteine, and N-acetyl-L-cysteine conjugates. Chem Res Toxicol 9(6):932-938.

Johnson EF. 2003. The 2002 Bernard B. Brodie Award lecture: deciphering substrate recognition by drug-metabolizing cytochromes P450. Drug Metab Dispos 31(12):1532-1540.

Joshi M, Tyndale RF. 2006a. Induction and recovery time course of rat brain CYP2E1 after nicotine treatment. Drug Metab Dispos 34(4):647-652.

Joshi M, Tyndale RF. 2006b. Regional and cellular distribution of CYP2E1 in monkey brain and its induction by chronic nicotine. Neuropharmacology 50(5):568-575.

Kang MY, Tsuchiya M, Packer L, Manabe M. 1998. In vitro study on antioxidant potential of various drugs used in the perioperative period. Acta Anaesthesiol Scand 42(1):4-12.

Kapitulnik J, Strobel HW. 1999. Extrahepatic drug metabolizing enzymes. J Biochem Mol Toxicol 13(5):227-230.

Karuzina, II, Archakov AI. 1994a. Hydrogen peroxide-mediated inactivation of microsomal cytochrome P450 during monooxygenase reactions. Free Radic Biol Med 17(6):557-567.

Karuzina, II, Archakov AI. 1994b. The oxidative inactivation of cytochrome P450 in monooxygenase reactions. Free Radic Biol Med 16(1):73-97.

Kerenyi L, Ricaurte GA, Schretlen DJ, McCann U, Varga J, Mathews WB, Ravert HT, Dannals RF, Hilton J, Wong DF, Szabo Z. 2003. Positron emission tomography of striatal serotonin transporters in Parkinson disease. Arch Neurol 60(9):1223-1229.

Kharasch ED, Hankins DC, Cox K. 1999. Clinical isoflurane metabolism by cytochrome P450 2E1. Anesthesiology 90(3):766-771.

Kharasch ED, Thummel KE. 1993. Identification of cytochrome P450 2E1 as the predominant enzyme catalyzing human liver microsomal defluorination of sevoflurane, isoflurane, and methoxyflurane. Anesthesiology 79(4):795-807.

Kharasch ED, Thummel KE, Mhyre J, Lillibridge JH. 1993. Single-dose disulfiram inhibition of chlorzoxazone metabolism: a clinical probe for P450 2E1. Clin Pharmacol Ther 53(6):643-650.

Kim H, Oh E, Im H, Mun J, Yang M, Khim JY, Lee E, Lim SH, Kong MH, Lee M, Sul D. 2006. Oxidative damages in the DNA, lipids, and proteins of rats exposed to isofluranes and alcohols. Toxicology 220(2-3):169-178.

Kim JH, Sherman ME, Curriero FC, Guengerich FP, Strickland PT, Sutter TR. 2004. Expression of cytochromes P450 1A1 and 1B1 in human lung from smokers, non-smokers, and ex-smokers. Toxicol Appl Pharmacol 199(3):210-219.

Kish SJ, Rajput A, Gilbert J, Rozdilsky B, Chang LJ, Shannak K, Hornykiewicz O. 1986. Elevated gamma-aminobutyric acid level in striatal but not extrastriatal brain regions in Parkinson's disease: correlation with striatal dopamine loss. Ann Neurol 20(1):26-31.

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. 1998. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392(6676):605-608.

Koivusalo M, Lindros KO. 1997. Phenethyl isothiocyanate as an inhibitor of aldehyde dehydrogenases. Adv Exp Med Biol 414:225-232.

Kolm RH, Danielson UH, Zhang Y, Talalay P, Mannervik B. 1995. Isothiocyanates as substrates for human glutathione transferases: structure-activity studies. Biochem J 311 (Pt 2):453-459.

Lagerkvist S. 1991. Sample splitting provides a fast and selective method for determining brain dialysate dopamine and its metabolites. In: Rollema H, Westerink BH, Drijhout WJ, editors. Monitoring molecules in neuroscience. Groningen, The Netherlands. p. 136.

Langston JW, Ballard PA, Jr. 1983. Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. N Engl J Med 309(5):310.

Leclercq I, Desager JP, Horsmans Y. 1998. Inhibition of chlorzoxazone metabolism, a clinical probe for CYP2E1, by a single ingestion of watercress. Clin Pharmacol Ther 64(2):144-149.

Lee AM, Miksys S, Palmour R, Tyndale RF. 2006. CYP2B6 is expressed in African Green monkey brain and is induced by chronic nicotine treatment. Neuropharmacology 50(4):441-450.

Leentjens AF. 2004. Depression in Parkinson's disease: conceptual issues and clinical challenges. J Geriatr Psychiatry Neurol 17(3):120-126.

Leroy E, Boyer R, Polymeropoulos MH. 1998. Intron-exon structure of ubiquitin c-terminal hydrolase-L1. DNA Res 5(6):397-400.

Lewy FH. 1914. Zur pathologischen Anatomie der paralysis agitans. Dtsch Z Nerveheilk 1:50-55.

Li AA, Mink PJ, McIntosh LJ, Teta MJ, Finley B. 2005. Evaluation of epidemiologic and animal data associating pesticides with Parkinson's disease. J Occup Environ Med 47(10):1059-1087.

Li XM, Juorio AV, Paterson IA, Walz W, Zhu MY, Boulton AA. 1992. Gene expression of aromatic L-amino acid decarboxylase in cultured rat glial cells. J Neurochem 59(3):1172-1175.

Lieber CS. 1997. Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev 77(2):517-544.

Limousin P, Martinez-Torres I. 2008. Deep brain stimulation for Parkinson's disease. Neurotherapeutics 5(2):309-319.

Lindner KJ, Hartvig P, Tedroff J, Ljungstrom A, Bjurling P, Langstrom B. 1995. Liquid chromatographic analysis of brain homogenates and microdialysates for the quantification of L-[beta-11C]DOPA and its metabolites for the validation of positron emission tomography studies. J Pharm Biomed Anal 13(4-5):361-367.

Lindros KO, Badger T, Ronis M, Ingelman-Sundberg M, Koivusalo M. 1995. Phenethyl isothiocyanate, a new dietary liver aldehyde dehydrogenase inhibitor. J Pharmacol Exp Ther 275(1):79-83.

Liu B, Gao HM, Hong JS. 2003. Parkinson's disease and exposure to infectious agents and pesticides and the occurrence of brain injuries: role of neuroinflammation. Environ Health Perspect 111(8):1065-1073.

Liu M, Liu S, Peterson SL, Miyake M, Liu KJ. 2002. On the application of 4-hydroxybenzoic acid as a trapping agent to study hydroxyl radical generation during cerebral ischemia and reperfusion. Mol Cell Biochem 234-235(1-2):379-385.

Loeper J, Descatoire V, Maurice M, Beaune P, Belghiti J, Houssin D, Ballet F, Feldmann G, Guengerich FP, Pessayre D. 1993. Cytochromes P-450 in human hepatocyte plasma membrane: recognition by several autoantibodies. Gastroenterology 104(1):203-216.

Logroscino G. 2005. The role of early life environmental risk factors in Parkinson disease: what is the evidence? Environ Health Perspect 113(9):1234-1238.

Ma Y, Dhawan V, Mentis M, Chaly T, Spetsieris PG, Eidelberg D. 2002. Parametric mapping of [18F]FPCIT binding in early stage Parkinson's disease: a PET study. Synapse 45(2):125-133.

Maher NE, Currie LJ, Lazzarini AM, Wilk JB, Taylor CA, Saint-Hilaire MH, Feldman RG, Golbe LI, Wooten GF, Myers RH. 2002a. Segregation analysis of Parkinson disease revealing evidence for a major causative gene. Am J Med Genet 109(3):191-197.

Maher NE, Golbe LI, Lazzarini AM, Mark MH, Currie LJ, Wooten GF, Saint-Hilaire M, Wilk JB, Volcjak J, Maher JE, Feldman RG, Guttman M, Lew M, Waters CH, Schuman S, Suchowersky O, Lafontaine AL, Labelle N, Vieregge P, Pramstaller PP, Klein C, Hubble J, Reider C, Growdon J, Watts R, Montgomery E, Baker K, Singer C, Stacy M, Myers RH. 2002b. Epidemiologic study of 203 sibling pairs with Parkinson's disease: the GenePD study. Neurology 58(1):79-84.

Mari M, Cederbaum AI. 2001. Induction of catalase, alpha, and microsomal glutathione S-transferase in CYP2E1 overexpressing HepG2 cells and protection against short-term oxidative stress. Hepatology 33(3):652-661.

Marklund N, Lewander T, Clausen F, Hillered L. 2001. Effects of the nitrone radical scavengers PBN and S-PBN on in vivo trapping of reactive oxygen species after traumatic brain injury in rats. J Cereb Blood Flow Metab 21(11):1259-1267.

Martignoni M, Groothuis GM, de Kanter R. 2006. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol 2(6):875-894.

Mattila KM, Rinne JO, Lehtimaki T, Roytta M, Ahonen JP, Hurme M. 2002. Association of an interleukin 1B gene polymorphism (-511) with Parkinson's disease in Finnish patients. J Med Genet 39(6):400-402.

McCarver DG, Byun R, Hines RN, Hichme M, Wegenek W. 1998. A genetic polymorphism in the regulatory sequences of human CYP2E1: association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. Toxicol Appl Pharmacol 152(1):276-281.

McGeer PL, Yasojima K, McGeer EG. 2002. Association of interleukin-1 beta polymorphisms with idiopathic Parkinson's disease. Neurosci Lett 326(1):67-69.

McLean KJ, Sabri M, Marshall KR, Lawson RJ, Lewis DG, Clift D, Balding PR, Dunford AJ, Warman AJ, McVey JP, Quinn AM, Sutcliffe MJ, Scrutton NS, Munro AW. 2005. Biodiversity of cytochrome P450 redox systems. Biochem Soc Trans 33(Pt 4):796-801.

Melega WP, Luxen A, Perlmutter MM, Nissenson CH, Phelps ME, Barrio JR. 1990. Comparative in vivo metabolism of 6-[18F]fluoro-L-dopa and [3H]L-dopa in rats. Biochem Pharmacol 39(12):1853-1860.

Mercer L, del Fiacco M, Cuello AC. 1979. The smooth endoplasmic reticulum as a possible storage site for dendritic dopamine in substantia nigra neurones. Experientia 35(1):101-103.

Mink JW. 1999. Basal Ganglia. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR, editors. Fundamental Neuroscience. San Diego, London: Academic Press. p. 951-972.

Modrek B, Lee C. 2002. A genomic view of alternative splicing. Nat Genet 30(1):13-19.

Moghaddam B, Bunney BS. 1989. Ionic composition of microdialysis perfusing solution alters the pharmacological responsiveness and basal outflow of striatal dopamine. J Neurochem 53(2):652-654.

Montoliu C, Sancho-Tello M, Azorin I, Burgal M, Valles S, Renau-Piqueras J, Guerri C. 1995. Ethanol increases cytochrome P4502E1 and induces oxidative stress in astrocytes. J Neurochem 65(6):2561-2570.

Moreno RL, Goosen T, Kent UM, Chung FL, Hollenberg PF. 2001. Differential effects of naturally occurring isothiocyanates on the activities of cytochrome P450 2E1 and the mutant P450 2E1 T303A. Arch Biochem Biophys 391(1):99-110.

Morens DM, Grandinetti A, Reed D, White LR, Ross GW. 1995. Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? Neurology 45(6):1041-1051.

Morris CR, Chen SC, Zhou L, Schopfer LM, Ding X, Mirvish SS. 2004. Inhibition by allyl sulfides and phenethyl isothiocyanate of methyl-n-pentylnitrosamine dependentylation by rat esophageal microsomes, human and rat CYP2E1, and Rat CYP2A3. Nutr Cancer 48(1):54-63.

Nakajima M, Yoshida R, Shimada N, Yamazaki H, Yokoi T. 2001. Inhibition and inactivation of human cytochrome P450 isoforms by phenethyl isothiocyanate. Drug Metab Dispos 29(8):1110-1113.

Nakamura K, Ahmed M, Barr E, Leiden JM, Kang UJ. 2000. The localization and functional contribution of striatal aromatic L-amino acid decarboxylase to L-3,4-dihydroxyphenylalanine decarboxylation in rodent parkinsonian models. Cell Transplant 9(5):567-576.

Negrusz A, Adamowicz P, Saini BK, Webster DE, Juhascik MP, Moore CM, Schlemmer RF. 2005. Detection of ketamine and norketamine in urine of nonhuman primates after a single dose of ketamine using microplate enzyme-linked immunosorbent assay (ELISA) and NCI-GC-MS. J Anal Toxicol 29(3):163-168.

Neve EP, Eliasson E, Pronzato MA, Albano E, Marinari U, Ingelman-Sundberg M. 1996. Enzyme-specific transport of rat liver cytochrome P450 to the Golgi apparatus. Arch Biochem Biophys 333(2):459-465.

Neve EP, Ingelman-Sundberg M. 2000. Molecular basis for the transport of cytochrome P450 2E1 to the plasma membrane. J Biol Chem 275(22):17130-17135.

Nicholl DJ, Bennett P, Hiller L, Bonifati V, Vanacore N, Fabbrini G, Marconi R, Colosimo C, Lamberti P, Stocchi F, Bonuccelli U, Vieregge P, Ramsden DB, Meco G, Williams AC. 1999. A study of five candidate genes in Parkinson's disease and related neurodegenerative disorders. European Study Group on Atypical Parkinsonism. Neurology 53(7):1415-1421.

Nichols WC, Elsaesser VE, Pankratz N, Pauciulo MW, Marek DK, Halter CA, Rudolph A, Shults CW, Foroud T. 2007. LRRK2 mutation analysis in Parkinson disease families with evidence of linkage to PARK8. Neurology 69(18):1737-1744.

Nicklas WJ, Vyas I, Heikkila RE. 1985. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. Life Sci 36(26):2503-2508.

Nicklas WJ, Youngster SK, Kindt MV, Heikkila RE. 1987. MPTP, MPP+ and mitochondrial function. Life Sci 40(8):721-729.

Nirenberg MJ, Chan J, Liu Y, Edwards RH, Pickel VM. 1996. Ultrastructural localization of the vesicular monoamine transporter-2 in midbrain dopaminergic neurons: potential sites for somatodendritic storage and release of dopamine. J Neurosci 16(13):4135-4145.

Nishikawa A, Furukawa F, Lee IS, Tanaka T, Hirose M. 2004. Potent chemopreventive agents against pancreatic cancer. Curr Cancer Drug Targets 4(4):373-384.

Nishina K, Akamatsu H, Mikawa K, Shiga M, Maekawa N, Obara H, Niwa Y. 1998. The inhibitory effects of thiopental, midazolam, and ketamine on human neutrophil functions. Anesth Analg 86(1):159-165.

Nissbrandt H, Bergquist F, Jonason J, Engberg G. 2001. Inhibition of cytochrome P450 2E1 induces an increase in extracellular dopamine in rat substantia nigra: a new metabolic pathway? Synapse 40(4):294-301.

Nissbrandt H, Carlsson A. 1987. Turnover of dopamine and dopamine metabolites in rat brain: comparison between striatum and substantia nigra. J Neurochem 49(3):959-967.

Nissbrandt H, Pileblad E, Carlsson A. 1985. Evidence for dopamine release and metabolism beyond the control of nerve impulses and dopamine receptors in rat substantia nigra. J Pharm Pharmacol 37(12):884-889.

Nissbrandt H, Sundstrom E, Jonsson G, Hjorth S, Carlsson A. 1989. Synthesis and release of dopamine in rat brain: comparison between substantia nigra pars compacts, pars reticulata, and striatum. J Neurochem 52(4):1170-1182.

Obeso JA, Olanow CW, Nutt JG. 2000. Levodopa motor complications in Parkinson's disease. Trends Neurosci 23(10 Suppl):S2-7.

Oinonen T, Mode A, Lobie PE, Lindros KO. 1996. Zonation of cytochrome P450 enzyme expression in rat liver. Isozyme-specific regulation by pituitary dependent hormones. Biochem Pharmacol 51(10):1379-1387.

Okulicz M, Bialik I, Chichlowska J. 2005. The time-dependent effect of gluconasturtiin and phenethyl isothiocyanate on metabolic and antioxidative parameters in rats. J Anim Physiol Anim Nutr (Berl) 89(11-12):367-372.

Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, Lopez de Munain A, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de Silva R, Lees A, Marti-Masso JF, Perez-Tur J, Wood NW, Singleton AB. 2004. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44(4):595-600.

Pankow D, Damme B, Schror K. 1994. Acetylsalicylic acid--inducer of cytochrome P-450 2E1? Arch Toxicol 68(4):261-265.

Park KS, Sohn DH, Veech RL, Song BJ. 1993. Translational activation of ethanol-inducible cytochrome P450 (CYP2E1) by isoniazid. Eur J Pharmacol 248(1):7-14.

Parkinson J. 1817. An Essay on the Shaking Palsy. London: Whitingham and Rowland.

Paxinos S, Watson C. 1986. The rat brain in stereotaxic co-ordinates. Sydney: Academic Press.

Pearce RK, Owen A, Daniel S, Jenner P, Marsden CD. 1997. Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. J Neural Transm 104(6-7):661-677.

Peeters EA, Bloem BR, Kuiper MA, Vermeij P, de Wolff FA, Wolters EC, Roos RA, Ferrari MD. 1994. Phenytoin parahydroxylation is not impaired in patients with young-onset Parkinson's disease. Clin Neurol Neurosurg 96(4):296-299.

Peng HM, Coon MJ. 1998. Regulation of rabbit cytochrome P450 2E1 expression in HepG2 cells by insulin and thyroid hormone. Mol Pharmacol 54(4):740-747.

Pericic D, Walters JR. 1976. Dopamine in substantia nigra and cortex after gamma-butyrolactone treatment. J Pharm Pharmacol 28(6):527-530.

Persson JO, Terelius Y, Ingelman-Sundberg M. 1990. Cytochrome P-450-dependent formation of reactive oxygen radicals: isozyme-specific inhibition of P-450-mediated reduction of oxygen and carbon tetrachloride. Xenobiotica 20(9):887-900.

Pezzoli G, Antonini A, Barbieri S, Canesi M, Perbellini L, Zecchinelli A, Mariani CB, Bonetti A, Leenders KL. 1995. n-Hexane-induced parkinsonism: pathogenetic hypotheses. Mov Disord 10(3):279-282.

Piccini P, Brooks DJ. 1999. Etiology of Parkinson's disease: contributions from 18F-DOPA positron emission tomography. Adv Neurol 80:227-231.

Pickel VM, Beckley SC, Joh TH, Reis DJ. 1981. Ultrastructural immunocytochemical localization of tyrosine hydroxylase in the neostriatum. Brain Res 225(2):373-385.

Pickel VM, Joh TH, Reis DJ. 1977. Regional and ultrastructural localization of tyrosine hydroxylass by immunocytochemistry in dopaminergic neurons of the mesolimbic and nigroneostriatal systems. Adv Biochem Psychopharmacol 16:321-329.

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276(5321):2045-2047.

Przedborski S, Jackson-Lewis V, Vila M, Wu DC, Teismann P, Tieu K, Choi DK, Cohen O. 2003. Free radical and nitric oxide toxicity in Parkinson's disease. Adv Neurol 91:83-94.

Pucak ML, Grace AA. 1994. Evidence that systemically administered dopamine antagonists activate dopamine neuron firing primarily by blockade of somatodendritic autoreceptors. J Pharmacol Exp Ther 271(3):1181-1192.

Reicks MM, Crankshaw DL. 1996. Modulation of rat hepatic cytochrome P-450 activity by garlic organosulfur compounds. Nutr Cancer 25(3):241-248.

Reinke LA, Kotake Y, Moore DR, Nanji AA. 1998. Free radical formation during ketamine anesthesia in rats: a cautionary note. Free Radic Biol Med 24(6):1002-1006.

Riedl AG, Watts PM, Brown CT, Jenner P. 1999. P450 and heme oxygenase enzymes in the basal ganglia and their roles in Parkinson's disease. Adv Neurol 80:271-286.

Riedl AG, Watts PM, Edwards RJ, Boobis AR, Jenner P, Marsden CD. 1996. Selective localisation of P450 enzymes and NADPH-P450 oxidoreductase in rat basal ganglia using anti-peptide antisera. Brain Res 743(1-2):324-328.

Roberts BJ, Shoaf SE, Jeong KS, Song BJ. 1994. Induction of CYP2E1 in liver, kidney, brain and intestine during chronic ethanol administration and withdrawal: evidence that CYP2E1 possesses a rapid phase half-life of 6 hours or less. Biochem Biophys Res Commun 205(2):1064-1071.

Rocca WA, McDonnell SK, Strain KJ, Bower JH, Ahlskog JE, Elbaz A, Schaid DJ, Maraganore DM. 2004. Familial aggregation of Parkinson's disease: The Mayo Clinic family study. Ann Neurol 56(4):495-502.

Ronis MJ, Johansson I, Hultenby K, Lagercrantz J, Glaumann H, Ingelman-Sundberg M. 1991. Acetone-regulated synthesis and degradation of cytochrome P450E1 and cytochrome P4502B1 in rat liver [corrected]. Eur J Biochem 198(2):383-389.

Ronis MJJ, Lindros KO, Ingelman-Sundberg M. 1996. The CYP2 Subfamily. Ioannides C, editor. Boca Raton: CRC Press.

Rosengren E. 1960. On the role of monoamine oxidase for the inactivation of dopamine in brain. Acta Physiol Scand 49:370-375.

Rutledge C, Johanson J. 1967. Metabolic pathways of dopamine and norepinephrine in rabbit brain in vitro. J Pharmacol Exp Ther 157:493-502.

Sabbagh N, Brice A, Marez D, Durr A, Legrand M, Lo Guidice JM, Destee A, Agid Y, Broly F. 1999. CYP2D6 polymorphism and Parkinson's disease susceptibility. Mov Disord 14(2):230-236.

Sanchez-Catalan MJ, Hipolito L, Guerri C, Granero L, Polache A. 2008. Distribution and differential induction of CYP2E1 by ethanol and acetone in the mesocorticolimbic system of rat. Alcohol Alcohol 43(4):401-407.

Santiago M, Westerink BH. 1991. Characterization and pharmacological responsiveness of dopamine release recorded by microdialysis in the substantia nigra of conscious rats. J Neurochem 57(3):738-747.

Santiago M, Westerink BH. 1992. Simultaneous recording of the release of nigral and striatal dopamine in the awake rat. Neurochem Int 20 Suppl:107S-110S.

Sarre S, Yuan H, Jonkers N, Van Hemelrijck A, Ebinger G, Michotte Y. 2004. In vivo characterization of somatodendritic dopamine release in the substantia nigra of 6-hydroxydopamine-lesioned rats. J Neurochem 90(1):29-39.

Sasame HA, Ames MM, Nelson SD. 1977. Cytochrome P-450 and NADPH cytochrome c reductase in rat brain: formation of catechols and reactive catechol metabolites. Biochem Biophys Res Commun 78(3):919-926.

Sato C, Morgan A, Lang AE, Salehi-Rad S, Kawarai T, Meng Y, Ray PN, Farrer LA, St George-Hyslop P, Rogaeva E. 2005. Analysis of the glucocerebrosidase gene in Parkinson's disease. Mov Disord 20(3):367-370.

Schapira AH. 2007. Treatment options in the modern management of Parkinson disease. Arch Neurol 64(8):1083-1088.

Scholtissen B, Verhey FR, Steinbusch HW, Leentjens AF. 2006. Serotonergic mechanisms in Parkinson's disease: opposing results from preclinical and clinical data. J Neural Transm 113(1):59-73.

Schulte T, Schols L, Muller T, Woitalla D, Berger K, Kruger R. 2002. Polymorphisms in the interleukin-1 alpha and beta genes and the risk for Parkinson's disease. Neurosci Lett 326(1):70-72.

Seliskar M, Rozman D. 2007. Mammalian cytochromes P450--importance of tissue specificity. Biochim Biophys Acta 1770(3):458-466.

Shahi GS, Das NP, Moochhala SM. 1990. Parkinson's disease and cytochrome P450: a possible link? Med Hypotheses 32(4):277-282.

Siegle I, Fritz P, Eckhardt K, Zanger UM, Eichelbaum M. 2001. Cellular localization and regional distribution of CYP2D6 mRNA and protein expression in human brain. Pharmacogenetics 11(3):237-245.

Singh M, Khan AJ, Shah PP, Shukla R, Khanna VK, Parmar D. 2008. Polymorphism in environment responsive genes and association with Parkinson disease. Mol Cell Biochem 312(1-2):131-138.

Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K. 2003. alpha-Synuclein locus triplication causes Parkinson's disease. Science 302(5646):841.

Smith CA, Gough AC, Leigh PN, Summers BA, Harding AE, Maraganore DM, Sturman SG, Schapira AH, Williams AC, et al. 1992. Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. Lancet 339(8806):1375-1377.

Smith TJ, Guo Z, Guengerich FP, Yang CS. 1996. Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by human cytochrome P450 1A2 and its inhibition by phenethyl isothiocyanate. Carcinogenesis 17(4):809-813.

Sofic E, Lange KW, Jellinger K, Riederer P. 1992. Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci Lett 142(2):128-130.

Sofic E, Paulus W, Jellinger K, Riederer P, Youdim MB. 1991. Selective increase of iron in substantia nigra zona compacta of parkinsonian brains. J Neurochem 56(3):978-982.

Sofic E, Riederer P, Heinsen H, Beckmann H, Reynolds GP, Hebenstreit G, Youdim MB. 1988. Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. J Neural Transm 74(3):199-205.

Sohda T, Shimizu M, Kamimura S, Okumura M. 1993. Immunohistochemical demonstration of ethanol-inducible P450 2E1 in rat brain. Alcohol Alcohol Suppl 1B:69-75.

Song BJ, Matsunaga T, Hardwick JP, Park SS, Veech RL, Yang CS, Gelboin HV, Gonzalez FJ. 1987. Stabilization of cytochrome P450j messenger ribonucleic acid in the diabetic rat. Mol Endocrinol 1(8):542-547.

Song BJ, Veech RL, Park SS, Gelboin HV, Gonzalez FJ. 1989. Induction of rat hepatic N-nitrosodimethylamine demethylase by acetone is due to protein stabilization. J Biol Chem 264(6):3568-3572.

Standaert DG, Young AB. 1996. Treatment of central nervous system degenerative disorders. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gilman A, editors. The pharmacological basis of therapeutics. New York: McGraw-Hill, cop.

Stasiecki P, Oesch F, Bruder G, Jarasch ED, Franke WW. 1980. Distribution of enzymes involved in metabolism of polycyclic aromatic hydrocarbons among rat liver endomembranes and plasma membranes. Eur J Cell Biol 21(1):79-92.

Staudt H, Lichtenberger F, Ullrich V. 1974. The role of NADH in uncoupled microsomal monoxygenations. Eur J Biochem 46(1):99-106.

Ste-Marie L, Boismenu D, Vachon L, Montgomery J. 1996. Evaluation of sodium 4-hydroxybenzoate as an hydroxyl radical trap using gas chromatography-mass spectrometry and high-performance liquid chromatography with electrochemical detection. Anal Biochem 241(1):67-74.

Ste-Marie L, Vachon L, Bemeur C, Lambert J, Montgomery J. 1999. Local striatal infusion of MPP+ does not result in increased hydroxylation after systemic administration of 4-hydroxybenzoate. Free Radic Biol Med 27(9-10):997-1007.

Stenstrom A, Hardy J, Oreland L. 1987. Intra- and extra-dopamine-synaptosomal localization of monoamine oxidase in striatal homogenates from four species. Biochem Pharmacol 36(18):2931-2935.

Sveinbjornsdottir S, Hicks AA, Jonsson T, Petursson H, Gugmundsson G, Frigge ML, Kong A, Gulcher JR, Stefansson K. 2000. Familial aggregation of Parkinson's disease in Iceland. N Engl J Med 343(24):1765-1770.

Takakubo F, Yamamoto M, Ogawa N, Yamashita Y, Mizuno Y, Kondo I. 1996. Genetic association between cytochrome P450IA1 gene and susceptibility to Parkinson's disease. J Neural Transm 103(7):843-849.

Tanaka K, Weihrauch D, Kehl F, Ludwig LM, LaDisa JF, Jr., Kersten JR, Pagel PS, Warltier DC. 2002. Mechanism of preconditioning by isoflurane in rabbits: a direct role for reactive oxygen species. Anesthesiology 97(6):1485-1490.

Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, Langston JW. 1999. Parkinson disease in twins: an etiologic study. Jama 281(4):341-346.

Teismann P, Schulz JB. 2004. Cellular pathology of Parkinson's disease: astrocytes, microglia and inflammation. Cell Tissue Res 318(1):149-161.

Thapliyal R, Maru GB. 2001. Inhibition of cytochrome P450 isozymes by curcumins in vitro and in vivo. Food Chem Toxicol 39(6):541-547.

The International HapMap Project. 2003. Nature 426(6968):789-796.

Thomas PE, Bandiera S, Reik LM, Maines SL, Ryan DE, Levin W. 1987. Polyclonal and monoclonal antibodies as probes of rat hepatic cytochrome P-450 isozymes. Fed Proc 46(8):2563-2566.

Tierney DJ, Haas AL, Koop DR. 1992. Degradation of cytochrome P450 2E1: selective loss after labilization of the enzyme. Arch Biochem Biophys 293(1):9-16.

Tindberg N, Baldwin HA, Cross AJ, Ingelman-Sundberg M. 1996. Induction of cytochrome P450 2E1 expression in rat and gerbil astrocytes by inflammatory factors and ischemic injury. Mol Pharmacol 50(5):1065-1072.

Tornwall M, Kaakkola S, Tuomainen P, Kask A, Mannisto PT. 1994. Comparison of two new inhibitors of catechol O-methylation on striatal dopamine metabolism: a microdialysis study in rats. Br J Pharmacol 112(1):13-18.

Torreilles F, Salman-Tabcheh S, Guerin M, Torreilles J. 1999. Neurodegenerative disorders: the role of peroxynitrite. Brain Res Brain Res Rev 30(2):153-163.

Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, Huang P. 2006. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. Cancer Cell 10(3):241-252.

Trétiakoff C. 1919. Contribution al'etude del'anatomie pathologique du locus niger de Soemmering avec quelques deductions relatives a la pathogenie des troubles du tonus musculaire et de la maladie de Parkinson.: These de paris.

Truan G, Cullin C, Reisdorf P, Urban P, Pompon D. 1993. Enhanced in vivo monooxygenase activities of mammalian P450s in engineered yeast cells producing high levels of NADPH-P450 reductase and human cytochrome b5. Gene 125(1):49-55.

Tsuneoka Y, Fukushima K, Matsuo Y, Ichikawa Y, Watanabe Y. 1996. Genotype analysis of the CYP2C19 gene in the Japanese population. Life Sci 59(20):1711-1715.

Turman CM, Hatley JM, Ryder DJ, Ravindranath V, Strobel HW. 2006. Alternative splicing within the human cytochrome P450 superfamily with an emphasis on the brain: The convolution continues. Expert Opin Drug Metab Toxicol 2(3):399-418.

Ubeda JV. 1998. Null hypothesis of husband-wife concordance of Parkinson's disease in 1,000 married couples over age 50 in Spain. Neuroepidemiology 17(2):90-95.

Umeno M, McBride OW, Yang CS, Gelboin HV, Gonzalez FJ. 1988. Human ethanol-inducible P450IIE1: complete gene sequence, promoter characterization, chromosome mapping, and cDNA-directed expression. Biochemistry 27(25):9006-9013.

Upadhya SC, Tirumalai PS, Boyd MR, Mori T, Ravindranath V. 2000. Cytochrome P4502E (CYP2E) in brain: constitutive expression, induction by ethanol and localization by fluorescence in situ hybridization. Arch Biochem Biophys 373(1):23-34.

Vaglini F, Pardini C, Viaggi C, Bartoli C, Dinucci D, Corsini GU. 2004. Involvement of cytochrome P450 2E1 in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease. J Neurochem 91(2):285-298.

Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW. 2004. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304(5674):1158-1160.

Viaggi C, Pardini C, Vaglini F, Corsini GU. 2006. Cytochrome P450 and Parkinson's disease: protective role of neuronal CYP 2E1 from MPTP toxicity. J Neural Transm Suppl(70):173-176.

Vieira I, Sonnier M, Cresteil T. 1996. Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. Eur J Biochem 238(2):476-483.

Wang J, Liu Z, Chan P. 2000. Lack of association between cytochrome P450 2E1 gene polymorphisms and Parkinson's disease in a Chinese population. Mov Disord 15(6):1267-1269.

Wang PC, Kuchel O, Buu NT, Genest J. 1983. Catecholamine glucuronidation: an important metabolic pathway for dopamine in the rat. J Neurochem 40(5):1435-1440.

Wang QJ, Li KZ, Yao SL, Li ZH, Liu SS. 2008. Different effects of isoflurane and sevoflurane on cytotoxicity. Chin Med J (Engl) 121(4):341-346.

Wang Y, Chen S, Yang D, Le WD. 2007. Stem cell transplantation: a promising therapy for Parkinson's disease. J Neuroimmune Pharmacol 2(3):243-250.

Watts PM, Riedl AG, Douek DC, Edwards RJ, Boobis AR, Jenner P, Marsden CD. 1998. Co-localization of P450 enzymes in the rat substantia nigra with tyrosine hydroxylase. Neuroscience 86(2):511-519.

Westerink BH, Korf J. 1976. Turnover of acid dopamine metabolites in striatal and mesolimbic tissue of the rat brain. Eur J Pharmacol 37(2):249-255.

Westerink BH, Spaan SJ. 1982. Estimation of the turnover of 3-methoxytyramine in the rat striatum by HPLC with electrochemical detection: implications for the sequence in the cerebral metabolism of dopamine. J Neurochem 38(2):342-347.

Wilson CJ, Groves PM, Fifkova E. 1977. Monoaminergic synapses, including dendro-dendritic synapses in the rat substantia nigra. Exp Brain Res 30(2-3):161-174.

Wirdefeldt K, Gatz M, Schalling M, Pedersen NL. 2004. No evidence for heritability of Parkinson disease in Swedish twins. Neurology 63(2):305-311.

Wood PL, Altar CA. 1988. Dopamine release in vivo from nigrostriatal, mesolimbic, and mesocortical neurons: utility of 3-methoxytyramine measurements. Pharmacol Rev 40(3):163-187.

Woodcroft KJ, Novak RF. 1997. Insulin effects on CYP2E1, 2B, 3A, and 4A expression in primary cultured rat hepatocytes. Chem Biol Interact 107(1-2):75-91.

Wu D, Cederbaum AI. 1992. Presence of functionally active cytochrome P-450IIE1 in the plasma membrane of rat hepatocytes. Hepatology 15(3):515-524.

Wu D, Cederbaum AI. 2001. Sodium salicylate increases CYP2E1 levels and enhances arachidonic acid toxicity in HepG2 cells and cultured rat hepatocytes. Mol Pharmacol 59(4):795-805.

Wu RM, Cheng CW, Chen KH, Shan DE, Kuo JW, Ho YF, Chern HD. 2002. Genetic polymorphism of the CYP2E1 gene and susceptibility to Parkinson's disease in Taiwanese. J Neural Transm 109(11):1403-1414.

Wu SJ, Ng LT, Lin CC. 2005. Effects of antioxidants and caspase-3 inhibitor on the phenylethyl isothiocyanate-induced apoptotic signaling pathways in human PLC/PRF/5 cells. Eur J Pharmacol 518(2-3):96-106.

Wu XJ, Hua X. 2007. Targeting ROS: selective killing of cancer cells by a cruciferous vegetable derived pro-oxidant compound. Cancer Biol Ther 6(5):646-647.

Yadav S, Dhawan A, Singh RL, Seth PK, Parmar D. 2006. Expression of constitutive and inducible cytochrome P450 2E1 in rat brain. Mol Cell Biochem 286(1-2):171-180.

Yang CS, Chhabra SK, Hong JY, Smith TJ. 2001. Mechanisms of inhibition of chemical toxicity and carcinogenesis by diallyl sulfide (DAS) and related compounds from garlic. J Nutr 131(3s):1041S-1045S.

Yu R, Mandlekar S, Harvey KJ, Ucker DS, Kong AN. 1998. Chemopreventive isothiocyanates induce apoptosis and caspase-3-like protease activity. Cancer Res 58(3):402-408.

Zand R, Nelson SD, Slattery JT, Thummel KE, Kalhorn TF, Adams SP, Wright JM. 1993. Inhibition and induction of cytochrome P4502E1-catalyzed oxidation by isoniazid in humans. Clin Pharmacol Ther 54(2):142-149.

Zhang Y, Kolm RH, Mannervik B, Talalay P. 1995. Reversible conjugation of isothiocyanates with glutathione catalyzed by human glutathione transferases. Biochem Biophys Res Commun 206(2):748-755.

Zhang Y, Tang L, Gonzalez V. 2003. Selected isothiocyanates rapidly induce growth inhibition of cancer cells. Mol Cancer Ther 2(10):1045-1052.

Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, Stoessl AJ, Pfeiffer RF, Patenge N, Carbajal IC, Vieregge P, Asmus F, Muller-Myhsok B, Dickson DW, Meitinger T, Strom TM, Wszolek ZK, Gasser T. 2004. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 44(4):601-607.