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Levodopa pharmacokinetics -from stomach to brain

A study on patients with Parkinson's disease

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Till farmor och farfar!
To grandma and grandpa!

The more I learn, the less I realize I know
Socrates

CONTENTS

ABSTRACT.....	1
SVENSK SAMMANFATTNING	2
LIST OF PAPERS.....	3
ABBREVIATIONS	4
INTRODUCTION.....	6
Parkinson's Disease.....	6
The Basal Ganglia.....	7
Treatment of Parkinson's Disease	10
<i>Levodopa</i>	10
<i>Levodopa in the periphery</i>	11
<i>Levodopa in the brain</i>	13
<i>Motor Complications</i>	14
<i>MAO-inhibitors</i>	16
<i>Dopamine Agonists</i>	17
<i>Deep Brain Stimulation</i>	17
Gastric Emptying	19
AIMS OF THE THESIS.....	20
METHODS	21
Microdialysis	21
Carbon-labeled Octanoic Acid Breath Test	28
Lumbar Puncture.....	28
Mass Spectrometry	28
High-performance Liquid Chromatography.....	29
Deep Brain Stimulation.....	30
Statistics	32
PATIENTS AND MATERIALS.....	33
Patient selection	33
Materials and Chemicals.....	34
REVIEW OF THE PAPERS AND MAIN RESULTS	36
DISCUSSION.....	47

CONCLUSIONS	52
ACKNOWLEDGEMENTS	54
REFERENCES	57

ABSTRACT

Parkinson's disease (PD) is one of the most common neurodegenerative disorders and it is caused by a loss of dopamine (DA) producing neurons in the basal ganglia in the brain. The PD patient suffers from motor symptoms such as tremor, bradykinesia and rigidity and treatment with levodopa (LD), the precursor of DA, has positive effects on these symptoms. Several factors affect the availability of orally given LD. Gastric emptying (GE) is one factor and it has been shown to be delayed in PD patients resulting in impaired levodopa uptake. Different enzymes metabolize LD on its way from the gut to the brain resulting in less LD available in the brain and more side effects from the metabolites. By adding dopa decarboxylase inhibitors (carbidopa or benserazide) or COMT-inhibitors (e.g. entacapone) the bioavailability of LD increases significantly and more LD can pass the blood-brain-barrier and be converted to DA in the brain. It has been considered of importance to avoid high levodopa peaks in the brain because this seems to induce changes in postsynaptic dopaminergic neurons causing disabling motor complications in PD patients. More continuously given LD, e.g. duodenal or intravenous (IV) infusions, has been shown to improve these motor complications. Deep brain stimulation of the subthalamic nucleus (STN DBS) has also been proven to improve motor complications and to make it possible to reduce the LD dosage in PD patients.

In this doctoral thesis the main purpose is to study the pharmacokinetics of LD in patients with PD and motor complications; in blood and subcutaneous tissue and study the effect of GE and PD stage on LD uptake and the effect of continuously given LD (CDS) on LD uptake and GE; in blood and cerebrospinal fluid (CSF) when adding the peripheral enzyme inhibitors entacapone and carbidopa to LD infusion IV; in brain during STN DBS and during oral or IV LD treatment.

To conclude, LD uptake is more favorable in PD patients with less severe disease and GE is delayed in PD patients. No obvious relation between LD uptake and GE or between GE and PD stage is seen and CDS decreases the LD levels. Entacapone increases the maximal concentration of LD in blood and CSF. This is more evident with additional carbidopa and important to consider in avoiding high LD peaks in brain during PD treatment. LD in brain increases during both oral and IV LD treatment and the DA levels follows LD well indicating that PD patients still have capacity to metabolize LD to DA despite probable pronounced nigral degeneration. STN DBS seems to increase putaminal DA levels and together with IV LD treatment also increases LD in brain possibly explaining why it is possible to decrease LD medication after STN DBS surgery.

SVENSK SAMMANFATTNING

Parkinsons sjukdom (PS) är en av de vanligaste s.k. neurodegenerativa sjukdomarna och orsakas av förlust av dopamin(DA)producerande nervceller i hjärnan. Detta orsakar motoriska symptom såsom skakningar, stelhet och förlångsammade rörelser. Levodopa (LD) är ett ämne, som kan omvandlas till DA i hjärnan och ge symptomlindring och det är oftast förstahandsval vid behandling av patienter med PS. Flera faktorer påverkar tillgängligheten av LD, bl.a. den hastighet som magsäcken tömmer sig med och denna verkar förlångsammad hos personer med PS vilket ger sämre tillgänglighet av LD i blodet och därmed i hjärnan. LD bryts även ner i hög grad av olika enzym ute i kroppen vilket leder till mindre mängd LD som hamnar i hjärnan och till fler nedbrytningsprodukter som orsakar biverkningar. Tillägg av enzymhämmare leder till ökad mängd LD som kan nå hjärnan och omvandlas till DA. Det anses viktigt att undvika höga toppar av LD i hjärnan då dessa verkar bidra till utvecklandet av besvärliga motoriska komplikationer hos patienter med PS. Om LD ges mer kontinuerligt, exempelvis som en kontinuerlig infusion in i tarmen eller i blodet, så minskar dessa motoriska komplikationer. Inopererande av stimulatorer i vissa delar av hjärnan (DBS) har också visat sig minska dessa motoriska komplikationer och även resultera i att man kan minska LD-dosen.

Huvudsyftet med den här avhandlingen är att studera LD hos patienter med PS; i blod och fettvävnad då LD ges i tablettform och se om det finns något samband med LD-upptag och hastigheten på magsäckstömningen (MT) och om kontinuerligt given LD påverkar LD-upptaget eller MT; i blod och i ryggmärgsvätska då enzymhämmarna entakapon och karbidopa tillsätts LD; i hjärna vid behandling med DBS och då LD ges både som tablett och som infusion i blodet.

Sammanfattningsvis kan vi se att LD-upptaget är mer gynnsamt hos patienter med PS i tidigare skede av sjukdomens komplikationsfas. MT är förlångsammad hos patienter med PS och det är inget tydligt samband mellan LD-upptag och MT eller mellan MT och sjukdomsgrad. Kontinuerligt given LD minskar LD-nivåerna. Enzymhämmaren entakapon ökar den maximala koncentrationen av LD i blod och ryggmärgsvätska och effekten är mer tydlig vid tillägg av karbidopa vilket är viktigt att ta i beaktande vid behandling av PS för att undvika höga toppar av LD i hjärnan. LD ökar i hjärnan då man behandlar med LD i tablettform och som infusion i blodet och DA-nivåerna i hjärnan följer LD väl vilket visar på att patienter med PS fortfarande kan omvandla LD till DA trots trolig uttalad brist av de DA-producerande nervcellerna i hjärnan. DBS verkar öka DA i vissa områden i hjärnan och tillsammans med LD-infusion i blodet verkar det även öka LD i hjärnan och det kan förklara varför man kan sänka LD-dosen efter DBS-operation.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to by their roman numerals:

I. M. Nord, A. Kullman, U. Hannestad, N. Dizdar

Is levodopa pharmacokinetics in patients with Parkinson's disease depending on gastric emptying?

Advances in Parkinson's disease. 2017, 6, 1-12

II. M. Nord, P. Zsigmond, A. Kullman, K. Arstrand, N. Dizdar

The effect of peripheral enzyme inhibitors on levodopa concentrations in blood and CSF

Movement Disorders. 2010 Feb 15;25(3):363-7

III. P. Zsigmond, M. Nord, A. Kullman, E. Diczfalusy, K. Wårdell, N. Dizdar

Neurotransmitter levels in basal ganglia during levodopa and deep brain stimulation treatment in Parkinson's disease

Neurology and Clinical Neuroscience. 2014, 2(5), 149-155

IV. M. Nord, P. Zsigmond, A. Kullman, N. Dizdar

Levodopa pharmacokinetics in brain after both oral and intravenous levodopa in one patient with advanced Parkinson's disease

Advances in Parkinson's disease. Accepted for publication 2017

ABBREVIATIONS

AADC	Aromatic amino acid decarboxylase
AUC	Area under the curve
BBB	Blood-brain-barrier
BG	Basal ganglia
CDS	Continuous dopaminergic stimulation
CI	Confidential interval
$C_{\max}$	Maximal concentration
$C_{\min}$	Minimum concentration
CNS	Central nervous system
COMT	Catechol- <i>O</i> -methyltransferase
CSF	Cerebrospinal fluid
CT	Computed tomography
DA	Dopamine
DAT	Dopamine transporter
DBS	Deep brain stimulation
DCAA	Aromatic amino acid decarboxylase
DDI	Dopa decarboxylase inhibitor
DOPAC	3, 4-Dihydroxy-phenylacetic acid
FEM	Finite element method
GABA	Gamma-aminobutyric acid
GE	Gastric emptying
GI	Gastrointestinal
Gp	Globus pallidus
GPe	Globus pallidus externa
Gpi	Globus pallidus interna
HFS	High frequent stimulation

HPLC	High-performance liquid chromatography
HVA	Homovanillic acid
IR	Infrared
IV	Intravenous
L-dopa	Levodopa (3, 4-dihydroxy-L-phenylalanine)
LID	Levodopa-induced dyskinesia
LNAA	Large neutral amino acids
MAO	Monoamine oxidase
MRI	Magnetic resonance imaging
MSN	Medium spiny neurons
3-MT	3-Methoxytyramine
NMS	Non-motor symptoms
PD	Parkinson's disease
PPN	Pedunculopontine nucleus
3-OMD	3- <i>O</i> -Methyldopa
SC	Subcutaneous
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
STN	Subthalamic nucleus
TH	Tyrosine hydroxylase
T _{1/2}	Half-time for gastric emptying, half-life for substances
T _{max}	Time to maximal concentration
UV	Ultraviolet
VA	Ventral anterior nucleus
VL	Ventral lateral nucleus
VMAT	Vesicular monoamine transporter
VTh	Ventroanterior and ventrolateral thalamus

INTRODUCTION

Parkinson's Disease

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. The overall prevalence of PD in persons above age 65 is app. 1.8 % and the prevalence of PD increases with age (1). The etiology still remains unknown but age, genetics and environmental factors influence. In PD there is a loss of dopamine (DA) producing neurons (dopaminergic neurons) in the basal ganglia (BG) of the brain (2). This results in the cardinal motor symptoms bradykinesia (slowed movements), distal tremor (shaking) and rigidity (stiffness). PD is a clinical diagnosis based on at least two of the three cardinal motor symptoms with asymmetrical onset and a positive response to levodopa or DA agonists. The most used system to describe the disease stages is according to the Modified Hoehn and Yahr staging:

- Stage 0: No signs of disease
- Stage 1: Unilateral disease
- Stage 1.5: Unilateral disease plus axial involvement
- Stage 2: Bilateral disease, without impaired balance
- Stage 2.5: Mild bilateral disease, with recovery on pull test
- Stage 3: Mild to moderate bilateral disease. Some postural instability.
Physically independent
- Stage 4: Severe disease. Still able to walk or stand unassisted
- Stage 5: Wheelchair bound or bedridden unless aided

However, in PD the peripheral and enteric nervous systems are also affected and PD patients also suffer from non-motor symptoms (NMS) such as depression, sleep disturbances, reduced olfaction and gastrointestinal (GI) symptoms such as obstipation (3-8). It seems as if especially vulnerable neurons are affected in a specific order in PD, according to the Braak model (9). In these neurons the protein α -synuclein aggregates to, so called Lewy bodies, seen in the cell bodies of the neurons and Lewy neurites in their neuronal processes. These inclusions affect the DA release negatively and the neurons eventually degenerate. According to the Braak model the neurons first affected are localized in the lower brainstem in the dorsal vagus nerve and in the anterior olfactory structures. The disease then progresses upwards through the medulla, pontine tegmentum, midbrain, basal forebrain and the cerebral cortex affecting more vulnerable neurons in these structures. It is possible that the PD progression is mediated by the spread and seeding of α -synuclein inclusions between neurons in a so called prion-like fashion (10, 11). PD pathology seems to start in the olfactory structures and the dorsal nerve and a hypothesis, the “dual theory”, is that an unknown neurotropic pathogen enters the nose and/or the gut and spreads via olfactory pathways and via the enteric plexus and preganglionic vagal fibers to the brain (12-14). This could explain why PD patients often suffer from olfactory and autonomic dysfunction early in disease, or even before PD diagnosis. It has been shown that total vagotomy results in a decreased PD risk, supporting the theory that the vagal nerve is involved in the pathogenesis of PD (15). In rat it has also been shown that α -synuclein can propagate from gut to brain (16) and this supports the theory that the Lewy body pathology spreads between neurons. Structures in the BG, involved in motor symptoms in PD, are examples of areas in the brain affected by the Lewy body pathology in PD.

The Basal Ganglia

The BG consists of substantia nigra (SN) pars compacta (SNc) and reticulata (SNr), striatum (putamen and caudate nucleus), globus pallidus (Gp) including the internal (Gpi) and external (Gpe) segments, nucleus accumbens and subthalamic nucleus (STN), **Figure 1**. Striatum together with globus pallidus constitute the corpus striatum. Putamen and Gp is called the nucleus lentiformis.

FUNCTIONAL DIVISIONS

1. **Striatum**
 - a. caudate nucleus
 - b. putamen
2. **Pallidum**
 - a. Globus Pallidus Interna (Gpi)
 - b. Globus Pallidus Externa (Gpe)
3. **Thalamus**
4. **Subthalamic Nucleus**
5. **Substantia Nigra**

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Figure 1. The basal ganglia.

Striatum has a central role in the BG collecting and processing information from the cerebral cortex and thalamus (17) and by serving as the primary input system to the BG. It consists of the putamen and the caudate nucleus divided by a white matter tract called capsula interna. In rodents about 95 % of the neurons in striatum are medium spiny neurons (MSN) (18) and they play an important role in movements, both initiating and controlling them. In the BG the most familiar neurotransmitters are gamma-aminobutyric acid (GABA) (inhibitory), glutamate (excitatory) and DA (inhibitory/excitatory). The neurotransmitter of MSN is GABA and it has an inhibitory effect on SNr and Gp to where the MSN project their axons. The MSN has DA receptors, some express D1-receptors and some express D2-receptors. Dopaminergic neurons project from SNc to putamen and DA excites the D1-type MSNs. These neurons send axons to Gpi/SNr which in turn projects to the ventroanterior and ventrolateral thalamus (VTh). From VTh neurons project to primary motor cortex. This is called the direct pathway and cortical activation of this pathway results in disinhibition of motor neurons allowing movements to take place. On the other hand DA from dopaminergic neurons from SNc inhibits the putaminal D2-type MSNs that project to Gpe. From Gpe axons project to STN. This is called the indirect pathway and cortical

activation of this pathway results in inhibition of motor neurons and difficulties to initiate movements. In the normal state, there is a delicate interaction between the direct and the indirect pathways in the fine tuning of movements. Impaired dopaminergic transmission in BG has been shown to be involved in causing motor symptoms in PD, **Figure 2**.

Even though we have been able to diagnose PD for decades and there are numerous theories of BG and its function we still know little about the mechanisms in the BG and its nuclei.

Figure 2. The basal ganglia network. A. Normal state. B. Parkinson's disease. **Direct pathway:** DA release from SNc activates D1 receptors and Gpi/SNr is then inhibited. This results in less inhibition of the thalamus which in turn results in activation of cortex. **Indirect pathway:** DA release from SNc activates D2 receptors and Gpe is then inhibited. This results in less inhibition of STN which has an activating effect on Gpi/SNr and therefore thalamus is inhibited resulting in less activation of cortex. Red arrow=inhibitory, green arrow=excitatory, grey arrow=DA transmission, black arrow=other neuronal networks, VA=ventral anterior nucleus, VL=ventral lateral nucleus, D1=D1-like DA receptors, D2=D2-like DA receptor, Gpe=Globus pallidus externa, Gpi=Globus pallidus interna, SNc=Substantia nigra pars compacta, SNr=Substantia nigra pars reticulata, STN=Subthalamic nucleus, PPN=Pedunculopontine nucleus.

Treatment of Parkinson's Disease

Levodopa

PD is a progressive disease and the focus of treatment is still primarily on symptom relief. It is not possible to give DA directly to PD patients because DA cannot cross the blood-brain-barrier (BBB). In the 1950s the Swedish scientist Arvid Carlsson and his co-workers saw that parkinsonian symptoms in animals decreased when given levodopa. Levodopa (L-dopa, L-3, 4-dihydroxy-phenylalanine) is a precursor to DA and, unlike DA, levodopa can cross the BBB. In brain the uptake and metabolism of levodopa to DA is mainly performed by dopaminergic neurons, which then release DA to the synaptic cleft, **Figure 3**.

Bravo *et al.* (2014).

DOI: 10.5772/57102. <http://www.intechopen.com/books/a-synopsis-of-parkinson-s-disease/pathophysiology-of-l-dopa-induced-dyskinesia-changes-in-d1-d3-receptors-and-their-signaling-pathway>

Figure 3. Synthesis of DA from levodopa in dopaminergic neuron. TH=Tyrosine hydroxylase, DCAA= Aromatic amino acid decarboxylase, DAT=DA transporter, D1=D1-like DA receptor, D2=D2-like DA receptor. VMAT=Vesicular monoamine transporter.

Levodopa has been used for several decades and orally given levodopa is still the gold standard, or even the platinum standard, in the treatment of patients with PD. New formulas have been developed and it is possible to give levodopa as standard, slow-release formulas and duodenal infusion, the two latter resulting in smoother levodopa concentrations in blood (19-23). There are several factors affecting the availability of levodopa on its way to the brain.

Levodopa in the periphery

Levodopa given orally or directly in the duodenum is absorbed in the proximal one-third of the small intestine and therefore the gastric emptying (GE) is one factor affecting the uptake of orally given levodopa (24). The GE has been shown to be delayed in PD patients (25-30) resulting in an impaired bioavailability of levodopa (31-34). Another factor is the competition between levodopa and other large neutral amino acids (LNAAs) that share the same active transport carrier system across the intestinal mucosa in the small intestine and a high amount of dietary proteins has been proposed to decrease the levodopa uptake (35-37).

In fasting PD patients with motor complications the levodopa absorption from the gut is rapid with time to peak plasma levodopa concentrations ($T_{\max}$) within 60 min (36, 38-40). The $T_{\max}$ increases threefold and the maximal concentration ($C_{\max}$) is decreased 30 % when levodopa is administrated after meal compared to fasting (38). Very low levels of orally administrated levodopa are excreted in faeces indicating a high absorption of levodopa (41, 42). When taken orally $C_{\max}$ of levodopa in blood is seen after 1-2 hours (41, 43) and levodopa is mainly excreted via the kidneys with two-thirds elimination within 8 hours (41) and 85 % elimination within 24 h (43). Only app. 1 % of levodopa, given as monotherapy, is eliminated in the urine unmetabolized, indicating a high peripheral metabolism (38, 42, 43). Levodopa is nearly undetectable in plasma 5 h after dosing.

Levodopa is mainly metabolized by the enzyme aromatic amino acid decarboxylase (AADC) and the enzyme catechol-*O*-methyltransferase (COMT), **Figure 4**, and app. 70 % of the levodopa is decarboxylated to DA and app. 10 % is *O*-methylated by COMT in the periphery (44). The result is less levodopa available to cross the intestinal muscosa and then to cross the BBB and higher levels of levodopa metabolites in the periphery causing adverse effects such as arrhythmias, orthostatic hypotension, nausea and vomiting. Orally administrated levodopa in human is mainly decarboxylated in the GI tract (45) and in brain capillary endothelium (46) but also in liver and kidney.

Figure 4. The metabolism of levodopa. COMT=Catechol-*O*-methyltransferase, AADC=Aromatic amino acid decarboxylase, 3-OMD=3-*O*-Methyldopa, MAO-B=Monoamine oxidase B, DOPAC=3, 4-Dihydroxy-phenylacetic acid, 3-MT=3-Methoxytyramine, HVA=Homovanillic acid.

It has been shown that the bioavailability of levodopa is higher in women compared to men (47, 48) and oestrogen is not the responsible factor (47). It is more likely the result of differences in COMT activity between gender (48). Age also seems to affect levodopa availability with higher levels in elder persons (age >65) and decarboxylation is thought to be the age dependent factor (49, 50).

In the 60-70s levodopa was given alone without any enzyme inhibitors and because of the enzyme degradation and the competition with other LNAAs across the intestinal mucosa, only 30 % of levodopa given orally reached the systemic circulation, i.e. the bioavailability was 30 % (22, 35). By adding an AADC inhibitor (DDI) to every levodopa dose the levodopa available in plasma is increased by 35-80 % in human (35, 49, 51-54). It has been shown that the DDI carbidopa reduces this first-pass metabolism to less than 10 % of the dose absorbed (52). The half-life ($T_{1/2}$) of levodopa is considered to be short and less than 60 min. However, previous studies have shown a range between 40-105 min when given as monotherapy (55-57) and between 60-95 min when adding a DDI (19, 35, 39).

In the treatment of PD levodopa is always given together with a DDI, either benserazide (Madopark[®]) or carbidopa (Sinemet[®]). However, when adding a

DDI, levodopa can then be metabolized to a higher extent by the COMT. With the addition of a peripheral COMT-inhibitor, such as entacapone to each levodopa/DDI dose, the levodopa concentration is increased between 23-40 % (58-61). Entacapone 200 mg has been shown to prolong the elimination $T_{1/2}$ of levodopa in blood with 26 % when levodopa is given together with DDI (carbidopa) (62).

Levodopa in the brain

When levodopa is transported across the BBB from blood to brain the same active transport carrier system as in the GI tract is used. Degradation of levodopa in the systemic circulation and competition with levodopa metabolites over the BBB results in lower levodopa levels in the brain. High protein-containing meals have also been shown to impair the clinical effect of levodopa and it might also be caused by the increased competition across the BBB where levodopa and other LNAAs share the same transport system (63-65). In the brain levodopa is metabolized to DA by AADC in dopaminergic neurons and the dopaminergic neurons autoregulate the release of DA in a healthy person resulting in smooth DA levels in the brain. The peripheral acting DDI-inhibitors and the COMT-inhibitor entacapone do not pass the BBB to any notable extent when given in therapeutical doses and therefore they do not affect the conversion of levodopa to DA in brain.

When giving levodopa/DDI to fasting patients in advanced PD the T_{max} occurred at 0.8 ± 0.1 h in plasma compared to 2.0 ± 0.4 h in cerebrospinal fluid (CSF) (40). Without an enzyme inhibitor only app. 1 % of the amount of levodopa, given orally is available to cross the BBB (38, 42, 43). The COMT-inhibitor entacapone has been shown to increase [^{18}F]-6-L-fluorodopa in striatum in PD patients with premedication with the DDI carbidopa (66). Several studies have stated that the addition of entacapone does not increase the C_{max} of levodopa (59, 60, 62, 67-70) in the periphery with the conclusion that the same applies for the brain.

In PD the considerable dopaminergic degeneration results in DA deficiency and by adding levodopa orally several times a day the deficiency is reduced. When adding levodopa with certain intervals the levodopa concentrations first increase and then decrease before the next tablet intake. PD patients eventually need increasing levodopa doses for symptom relief. Higher levodopa doses result in higher peaks (C_{max}) of levodopa concentration and this, together with the dopaminergic degeneration in the brain, is considered to result in motor complications. It is considered that with pronounced degeneration of the dopaminergic neurons it is possible that other structures, such as serotonergic neurons and/or astrocytes, are involved in the metabolism of levodopa to DA (71-77).

There is an increasing awareness that high levodopa peaks in the brain, together with the degeneration of dopaminergic neurons, is unfavorable in the development of motor complications in PD patients. When using peripheral enzyme inhibitors and other PD medications, such as monoamine oxidase (MAO) inhibitors and DA agonists, (see these specific sections), it is possible to use lower levodopa doses avoiding the high peaks of levodopa concentration. Unfortunately, most PD patients eventually experience motor complications.

Motor Complications

In the early stages of the disease levodopa in tablet form is an effective treatment and PD patients usually experience the so called “honeymoon period”. The longer the disease progresses, the more motor complications arise. At first, PD patients experience a predictable loss of the anti-parkinsonian effect of levodopa, the so-called end of dose or wearing off phenomenon, **Figure 5**. Wearing off phenomenon, seen in app. 50 % of PD patients the first year of levodopa treatment and in 100 % of the patients within 5 years (78), is thought to be caused by the progressive loss of dopaminergic presynaptic terminals in the SN resulting in a reduced ability to buffer the shifts in levodopa concentration due to intermittent oral administration of levodopa (79, 80). However, PD patients treated with apomorphine and other DA agonists, that are not stored in dopaminergic terminals, also suffer from wearing off (81). This indicates that postsynaptic mechanisms might be involved in the wearing off phenomenon (82, 83). PD patients with wearing off phenomenon need to take levodopa medication more frequently and therefore the daily levodopa dose increases. A higher daily levodopa dose results in higher levodopa concentrations and also higher levodopa peaks resulting in more adverse effects such as dyskinesia and the patient starts to experience the so called on-off syndrome. At first it is predictable with “on” periods, with troublesome dyskinesia, when the levodopa concentrations exceed the therapeutic window and “off” periods with severe parkinsonism, when the levodopa concentrations decline the therapeutic window, **Figure 5**. Later the PD patient experiences a more unpredictable and more abrupt loss of the drug effect with “on” and “off” periods without any relation to the levodopa intake.

Figure 5. The top diagram showing early disease, the middle diagram showing the principles of wearing off phenomenon and the bottom diagram showing predictable on-off syndrome with intermittent levodopa treatment. The therapeutic window for levodopa narrows during the progress of PD (84).

Dyskinesia in PD patients treated with levodopa are referred to as levodopa-induced dyskinesia (LID) with the three primary clinical syndromes peak-dose dyskinesia, diphasic dyskinesia and off-period dystonia (85). Within 5 years of treatment with levodopa 40-50 % of the PD patients experience LID (86-88). The underlying mechanisms for LID are still not fully understood but both presynaptic and postsynaptic changes in the nigro-striatal circuitry might be involved. The presynaptic mechanisms are caused by the DA neuron degeneration and there is a 70-80 % depletion of DA when PD patients start to

experience PD symptoms. Serotonergic neurons are able to convert exogenous levodopa to DA and release it as a “false transmitter” giving symptom relief in PD patients (71-75) and even though the serotonin innervation in the striatum also is affected in PD, it is not degenerated to the same extent as for the dopaminergic neurons (89). Serotonergic neurons may therefore play a role in the converting process of exogenous levodopa to DA. One theory is that the autoregulating function of the DA release is lacking in serotonergic neurons resulting in an un-controlled DA release after levodopa administration resulting in pulsatile stimulation of the striatal postsynaptic dopaminergic receptors and thus causing LID (90-92). There are also several NMS in PD, for example impaired cognition, depression and anxiety (8, 93-98) and the serotonergic hyperinnervation and the dysregulated DA release in different areas of the brain could be possible actors in the origination of some of them (93, 99, 100).

The pulsatile stimulation of striatal postsynaptic dopaminergic receptors caused by dopaminergic neuron degeneration, intermittent levodopa treatment causing high levodopa peaks (84, 85, 101, 102) and possible involvement of other neurons/astrocytes that convert levodopa to DA, seem to result in modifications of the postsynaptic dopaminergic receptors. It has been suggested that the pulsatile stimulation results in a sensitized signaling of DA receptors (D1-like and D2-like) resulting in abnormal signaling along different intracellular pathways resulting in changes in proteins and genes resulting in LID. It has also been shown that acute activation of the D1 receptor with levodopa, endogenously released DA or an intrastriatal full D1 receptor agonist (but not with the DA agonist ropinirole that binds to non-D1 receptors), results in internalization of the D1 receptors from the membrane to the cytoplasm (103, 104). In control subjects, with no neurological or psychiatric disease, it has been shown that D1 receptors are mostly detected on the membrane of MSNs, while there is a higher amount of intracellular D1 receptors in PD patients treated with levodopa (105). The theories of development of LID are reviewed extensively by Bastide *et al.* and Bezard *et al.* (106, 107). Motor complications are reduced when levodopa is given continuously (20, 108-110), resulting in a more continuous stimulation of the dopaminergic receptors. Previous studies have shown that continuous dopaminergic stimulation (CDS) seems to induce plasticity changes of the dopaminergic postsynaptic receptors reducing motor complications (20, 111, 112) and possibly restoring the receptor changes caused by previous PD medication.

MAO-inhibitors

The mitochondrial enzymes MAO A and B are involved in the oxidative deamination of different substances in the brain, for example the neurotransmitters serotonin and DA. DA is degraded by both MAO-A and MAO-B but mostly by MAO-B in human. By adding a MAO-B inhibitor more DA becomes available in the brain reducing motor symptoms in PD patients. MAO-B

inhibitors are given orally and they are used both as monotherapy and as complement to other PD medications. It has been considered that MAO-inhibitors are neuroprotective because they decrease the possible toxic byproducts by the MAO mediated reactions. However, there might be other neuroprotective effects such as antiapoptotic activity of some MAO-B inhibitors (113, 114). The most common side effects of MAO-B inhibitors are constipation, mild nausea, dry mouth and confusion and hallucinations in elder patients.

Dopamine Agonists

The DA agonist mimics the effects of DA by acting directly on DA receptors, both pre- and postsynaptic and was first introduced in 1974. DA agonists are available in an oral, transdermal and as a self-injectable form. An advantage of DA agonists is for example that they seem to result in fewer motor complications compared to levodopa (115). DA agonists are often considered the first choice of drug in young newly debuted PD patients in an attempt to postpone levodopa treatment and trying to delay the development of motor complications. They are also used later in disease as a complement to levodopa/DDI treatment in an attempt to reduce the levodopa dose and possibly have a positive effect on reducing LID (116). DA agonists do not need carrier-mediated transport over the intestinal mucosa or over the BBB, they do not need to be metabolized or stored before acting on the dopaminergic receptors and they give a more continuous stimulation of the receptors compared to levodopa because of its longer $T_{1/2}$. However, there are also adverse effects, for example valvular heart disease and compulsive behavior such as uncontrolled gambling and shopping and this can limit the clinical use of DA agonists (115, 117-119).

Deep Brain Stimulation

Deep brain stimulation (DBS) is a neurosurgical technique used in the treatment of for example essential tremor, primary dystonia, obsessive-compulsive disorder, chronic pain, and depression (120). However, DBS is most widely used in PD with disabling tremor, rigidity, bradykinesia and impaired gait and STN DBS was approved for treatment of PD in 2002. DBS can be used when PD medications do not have fulfilling effect. One or two electrodes are placed in certain areas of the BG and from the electrode a wire is led under the skin to a neurostimulator placed below the clavicle, **Figure 6**. The neurostimulator generates electrical impulses to the electrode. Placement of the electrode in the thalamus mostly reduces different kinds of tremor. When placed in the Gpi dystonia and all motor symptoms in PD are eased but the procedure does not result in reduced dose of PD medication. DBS in the STN has been shown effective both in reducing motor symptoms and in decreasing the daily doses of dopaminergic drugs in PD patients (121-127) and these effects seem to be maintained even several years after the surgical intervention (127-129). The mechanisms of DBS are still unclear (130) but there are several theories about

the action of DBS. An early hypothesis is the “depolarization blockade hypothesis” where DBS is proposed to inhibit neuronal activity in the stimulated area (125) and it might inhibit the production or release of certain neurotransmitters (131), mimicking a surgical lesion. Another theory is the “output activation hypothesis” where DBS is thought to induce action potentials in the axons resulting in an increasing output from the stimulated area. A third theory is that DBS activate fiber tracts surrounding the stimulated area and an example is activation of the nigro-striatal tract during STN stimulation showing significant increase of DA (132-134). Another theory about the mechanism(s) of DBS is that the high-frequency stimulation (HFS) during DBS regulates the pathological activity in the targeted area resetting the oscillatory patterns, so called “jamming” of neural activity (135).

In study III STN DBS was performed. During this study one patient received oral levodopa treatment on several occasions, out of protocol, before the start of STN DBS. This became an opportunity for us to investigate how oral intake of levodopa affects the levodopa and DA levels in a PD patient (paper IV), since similar data are sparse in literature from human BG *in vivo*.

Figure 6. The principles of STN DBS.

Gastric Emptying

As mentioned, several factors are affecting the availability of levodopa. Orally given levodopa is absorbed in the proximal one-third of the small intestine and, except for the competition between levodopa and other LNAAs across the intestinal mucosa (35, 36), GE is an important factor (24). It has been shown that more than 70 % of PD patients suffer from impaired gastric motility (27, 31) with symptoms like early satiety, postprandial bloating and nausea and it has been suggested that this is caused by the delay in GE that has been shown in PD patients (25-30, 136-138). Delayed GE has also been shown to impair the levodopa uptake and is therefore suggested to worsen motor complications (31-34, 136).

It is the autonomic and enteric nervous systems that mainly control the stomach functions and the dorsal motor nucleus of the vagal nerve is involved. This nucleus seems to be affected in PD (139) and within the enteric nervous system in the gut abnormalities, such as loss of dopaminergic neurons and Lewy bodies, have also been shown (140, 141). As mentioned earlier, a hypothesis is that PD pathology starts in the gut and spreads towards the central nervous system (CNS) (136). Obstipation is frequent in PD patients and there are indications of an elevated risk of future PD in people with infrequent bowel movement (142).

There is no conclusive data about the relation between delayed GE and PD stage. Some studies have shown that delayed GE in PD patients is associated with disease severity (31, 143) while other studies cannot find any association of GE and disease duration or Hoehn and Yahr clinical scale (25, 27, 28). Abnormalities in gastric motility have also been shown with electrogastrography in PD patients both in early and advanced stages of the disease (144, 145). It is possible that there is no strict relation between severity of motor symptoms and degree of impairment of GE in PD patients.

Different methods can be used for evaluating the GE rate in humans. The scintigraphic method has been a standard method but this demands that the patient lies completely still and therefore it has been difficult or impossible to examine PD patients suffering from dyskinesia and severe tremor. The octanoic acid breath test, based on the stable isotope ^{13}C in octanoic acid is another method for evaluating the GE and this method was used in paper I.

AIMS OF THE THESIS

Specific aims – paper I-IV:

I.

The aims of this paper were to investigate the levodopa uptake from the GI tract in PD patients with motor complications and to see if there is a correlation between levodopa uptake and GE and also to see if CDS, given as a continuous IV levodopa infusion for 10 days, affects the levodopa uptake and/or the GE.

II.

The aims of this paper were to investigate levodopa in blood and in CSF and to see what proportion of levodopa passing over the BBB. We also wanted to study the effects of the enzyme inhibitors entacapone and carbidopa on the levodopa concentrations in blood and CSF.

III.

The aims of this paper were to investigate the levels of different neurotransmitters with microdialysis technique in the brain, with and without STN DBS treatment, and to study the effect of STN DBS on levodopa treatment.

IV.

The aims of this paper were to investigate the levodopa levels in brain from one PD patient receiving oral levodopa and to compare these data with levodopa given intravenously (IV).

METHODS

Microdialysis

Microdialysis is a method that allows continuous sampling of unbound concentrations of both endogenous and exogenous substances (analytes) in different tissues, for example blood, subcutaneous (SC) tissue and brain (146). The technique is based on passive concentration dependent diffusion of substances over a semipermeable membrane (147, 148). Microdialysis can therefore also be used for delivering substances via the dialysate to the periprobal fluid, so called retrodialysis. However, here the focus will be on microdialysis as a sampling technique.

A primitive version of microdialysis was first described in 1958 by Kalant (149). In 1974 Ungerstedt and Pycocock used microdialysis for measuring neurotransmitters in rat brain and the microdialysis technique of today is based on their method (150). A Swedish research group improved the method in the early 80s by combining the use of small diameter dialysis tubes with sensitive high-pressure liquid chromatography (HPLC) analytical techniques (151). The microdialysis method in brain has continued to improve (152-158). In 1987 Lönnroth *et al.* showed that microdialysis is a useful method for measuring intercellular concentrations of different substances in SC tissue in human (159) and in 2007 probes for IV microdialysis became commercially available (160). It is now possible to examine many different tissues and microdialysis is used in both research and in monitoring treatments.

The principles of microdialysis

To use microdialysis, a microdialysis probe, a pump and a microvial to collect microdialysate, is necessary, **Figure 7**. The microdialysis probe is attached to a portable pump and a perfusate fluid is pumped through the probe. The inner diameter of the probe usually ranges between 0.15-0.3 mm and a semipermeable membrane is located at the tip of the probe.

Figure 7. Microdialysis system for IV and stereotactic CNS use; (1) Perfusion pump with perfusion fluid. (2) Microvials. (3) Probes for stereotactic CNS (at sign) and IV (above sign) use. (4) Semipermeable membrane pre-labelled with a golden tip.

When the fluid reaches the semipermeable membrane, substances from the perfusate and from the interstitial fluid moves along their concentration gradients finding equilibrium, **Figure 8**. The dialysate is then collected in the microvial. The concentration of the substance measured in the dialysate therefore reflects its concentration in the interstitial fluid. The rate of the perfusate flow is constant and is usually set between 0.3 to 3 $\mu\text{L}/\text{min}$ and factors to take into consideration when deciding the flow rate is the collected sample volume, how often samples are collected and analytical sensitivity needed. Sample collection time often ranges from 1 to 20 min (161).

Figure 8. Schematic illustration of a microdialysis probe.

It is possible to choose different cut-offs for the semipermeable membrane of the probe, depending on what substances are measured, and it usually range between 6 to 100 kDa. Substances with lower molecule weight than the cut-off are capable to pass over the membrane. Choosing a membrane with a lower cut-off, large molecules such as proteins are unable to cross the membrane, resulting in a protein free dialysate. This means that the dialysate contains only the free fraction of the substance of interest which is often preferable since the measured substance is in its active form when unbounded to proteins.

There are different types of probes, for example linear and concentric probes. The linear probe has a membrane imbedded within a length of small diameter tubing and is usually used for microdialysis in for example skin, SC tissue, muscle, liver and lung, **Figure 9a**. The most common concentric probe is the pin-styled where the membrane is located at the distal end of the supporting shaft. The rigid type is mainly used in preclinical studies, **Figure 9b**, and the flexible is used in peripheral veins and human brain, **Figure 9c**. It is also possible to choose different length of the probe depending on in what tissue microdialysis is used.

With permission from CMA Microdialysis AB

Figure 9. Different microdialysis probes; **a.** Linear (CMA 30 Linear Probe), **b.** Rigid pin-styled (CMA 11 MD Probe), **c.** Flexible pin-styled (CMA 20 MD Probe).

Recovery

When analyzing the results using microdialysis technique, it is of importance to evaluate the diffusion of the substances of interest over the membrane and recovery is then used.

Relative recovery

Relative recovery (concentration recovery) describes the ratio between the concentration of a substance in the outflow fluid and in the periprobe fluid and is presented as a ratio or a percentage. Relative recovery can be determined by placing the probe in a standardized solution containing a known concentration of a certain substance (matrix) and starting a constant perfusion rate of the probe with a fluid without that substance. The relative recovery is then calculated as:

$$\text{relative recovery}_{(\text{in vitro})} = C_d/C_m,$$

where C_d is the concentration in the dialysate and C_m the concentration in the matrix. Slow perfusion rate allows more time for the substance to find equilibrium over the membrane compared to high perfusion speed. Slower perfusion rate therefore gives higher concentration of the substance in the dialysate and a higher relative recovery. Having decided the relative recovery *in vitro* it is possible to get an estimated concentration of the substance in the periprobe fluid, for example in brain interstitial space: $C_{(\text{brain})} = C_d / \text{recovery}_{(\text{in vitro})}$. However, this formula often underestimates the true interstitial concentration of a substance.

Absolute recovery

Absolute recovery (mass recovery) describes the total amount of the substance of interest collected in the dialysate during a defined time period. The relative recovery is often of more interest than the absolute. However, if the concentration of the substance is low in the interstitial space and the substance is removed by microdialysis, it might alter the physiological process studied.

Recovery is affected by several factors such as;

- **Membrane area and its characteristics.**

Increased membrane area results in increased relative and absolute recoveries. Different materials of the membrane may affect the transport of the substance over the membrane. The most commonly used membrane materials are polycarbonate ether, regenerated cellulose and polyacrylonitrile and they have different properties. The membrane should be chosen according to the tissue and purpose of the investigation (162).

- **Perfusion flow rate.**

Slow perfusion rate allows more time for the substance to find equilibrium over the membrane compared to high perfusion speed. Slower perfusion rate therefore gives higher concentration of the substance in the dialysate and a higher relative recovery. In contrast to the relative recovery, the absolute recovery increases as perfusion rate increase. This also means higher dialysate volume and more diluted perfusate.

- **Start of perfusion.**

When inserting the microdialysis probe the damage of the tissue results in leakage of substances from the tissue. This may affect the diffusion of the substance of interest and therefore also the recovery. It is of importance to wait before starting the perfusion after inserting the probe and the optimal times after probe implantation differs between different tissues and substances of interest (158, 163).

- **Concentration of the substance of interest.**

Absolute recovery is dependent of the periprobal concentration of the substance of interest while the relative recovery is independent of it.

- **Temperature.**

In general, increased temperature may lead to higher relative recovery (155, 158, 164). It is therefore recommended to perform probe recovery *in vitro* at the same temperature as the tissue (165-167).

- **Perfusate.**

It is preferable to have a perfusate with similar properties (pH, ion strength, osmotic value) as the extracellular fluid of the dialyzed tissue.

- **Tissue properties.**

The matrix tortuosity affects the diffusion of the analyte. Higher tortuosity impedes the analyte diffusion through the tissue and therefore results in poor *in vivo* probe recovery. Adding an antithrombotic agent to the perfusate is recommended to avoid clotting of the membrane.

Advantages with microdialysis

The advances of microdialysis towards for example traditional blood sampling is that the microdialysate sample is protein free and only the free fraction of the actual substance is measured, no further clean-up is needed and enzymatic degradation is of no concern. Microdialysis also allows continuous sampling during longer periods of time like hours, days or even weeks and the samples can be collected in small fractions à 15 min per fraction and this without any fluid loss at the sampling site. Microdialysis is often a lenient sampling method for the patient.

Limitations with microdialysis

Even though microdialysis is a lenient sampling method in many aspects, the tissue penetrated by the microdialysis probe is damaged in some way. When placing a probe in peripheral veins the damage is possibly less than for example in brain tissue where the probe often is placed deeper in the tissue and also may affect the BBB. Another limitation with microdialysis is that it does not allow detection of rapid changes in concentrations of substances because of relatively long sampling periods. To determine the recovery of the probe can also be a limitation of the method; see the above section about relative recovery.

Areas of application

Microdialysis is, as mentioned above, possible to use in many tissues, such as brain, SC tissue, blood but also in for example liver, skin and heart muscle (146). Microdialysis is also used in intensive care allowing continuous monitoring of energy metabolites in different organs (168). In the last decades microdialysis in the human brain has allowed us to study neurotransmitters in different conditions, for example in PD. In 1990 the first study using stereotaxy in combination with microdialysis in human was published (169) and after that several studies have shown that microdialysis is a feasible method for studying neurotransmitter levels in the BG in human (130, 170-172).

In conclusion, microdialysis is a sampling method suitable for sampling over long periods of time without blood loss and it is often a lenient method for the patient. Microdialysis has evolved to a sampling technique that allows monitoring of the free fraction of endogenous as well as exogenous substances in almost any tissue.

In all papers, included in this doctoral thesis, we have used microdialysis as a sampling method. In paper I the microdialysis probe for SC tissue was commercially available, in paper I-II the IV probes were custom made and in paper III-IV the probes for stereotactic use were custom made and the IV probe was commercially available.

In paper I microdialysis was used in blood and SC where SC tissue is more lipophilic compared to blood and therefore may reflect the properties of brain tissue better. The flow rate for IV microdialysis was set at 1.0 $\mu\text{L}/\text{min}$ and the dialysate fractions were 15 μL and collected every 15 min for 4 h. The flow rate for SC microdialysis was set for 0.5 $\mu\text{L}/\text{min}$ and the dialysate fractions were 15 μL and collected every 30 min for 4 h. Levodopa was measured in the dialysates. Mean *in vitro* recovery was 77.1 % and 77.9 % for the IV and SC probes respectively.

In paper II microdialysis was used in blood, the flow rate was set for 1.0 $\mu\text{L}/\text{min}$ and the dialysate fractions (30 μL) were collected every 30 min for 12 h. Mean *in vitro* recovery for the IV probes was 56.6 %.

In paper III and IV microdialysis was used in blood and thanks to interested neurosurgeons and helpful PD patients we were able to investigate levodopa and its conversion to DA in brain tissue. The flow rate was set at 0.5 $\mu\text{L}/\text{min}$ and the microdialysate sampling started 3–4 h after STN DBS surgery. Sampling time was 1 h/fraction (15 μL) daytime and 2 h/fraction (60 μL) during the night. The flow rate for IV microdialysis was set at 1.0 $\mu\text{L}/\text{min}$ and the dialysate fractions (60 μL) were collected every hour during levodopa infusion period. In paper III mean *in vitro* recovery for brain and IV probes were 91 and 88 % respectively. In paper IV the recovery for brain probes was 65.7 % in left Gpi and 75.1 % in right Gpi. The probe from right putamen was damaged and the recovery process was not possible to perform. Recovery for the IV probe was 65.6 %. In paper III-IV STN DBS was performed according to a specific protocol and the effect of STN DBS on levodopa and DA was also possible to study with microdialysis.

Carbon-labeled Octanoic Acid Breath Test

In paper I we used the carbon-labeled octanoic acid breath test by Ghooos *et al.* (173), shown to be a reliable and safe method for evaluating the GE rate and suitable for patients in all stages of PD (143, 174, 175). ^{13}C marked octanoic acid is preserved in the solid phase of egg yolk used as standard meal. A rapid disintegration of the solid phase takes place in the duodenum where the ^{13}C marked octanoic acid is absorbed through the intestinal mucosa. The ^{13}C marked octanoic acid is then oxidized to $^{13}\text{CO}_2$ in the liver and then transported to the lungs and exhaled in breath together with ordinary $^{12}\text{CO}_2$. The absorption and oxidation of ^{13}C marked octanoic acid do not seem to affect the rate of $^{13}\text{CO}_2$ excretion in breath while the rate of GE of the egg yolk to duodenum is the rate limiting step making the test suitable for evaluating GE rate (173).

In paper I all patients were given an omelet containing a standardized amount of protein and ^{13}C marked octanoic acid after an overnight fast. Together with the omelet the patients were given 1 tablet of Madopark[®] (100 mg of levodopa/25 mg of benserazide). During a period of 4 h IV dialysate fractions and breath samples were taken every 15-min and SC dialysate fractions were collected every 30-min. The breath test was performed with the patient in an upright sitting position exhaling in a 10 mL Vacutainer[®] for app. 2 seconds and the tube was then immediately sealed with a rubber stopper. The breath samples were stored in room temperature and the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio was analyzed with mass spectrometry (173). $T_{1/2}$, which represents the half-time of $^{13}\text{CO}_2$ elimination from stomach, was calculated. Every experiment took app. 5 h.

Lumbar Puncture

In paper II a neurosurgeon performed lumbar puncture on the patients and an intradural catheter (63 cm) with a three-way-tap was placed in the lumbar region. 2-mL samples of CSF were taken hourly for 12 h. It is not possible to measure DA within CSF because of the quick degradation of DA. We therefore only studied the levodopa levels to investigate its passage over the BBB from blood.

Mass Spectrometry

Mass spectrometry is an analytical method where individual molecules are converted to ions. The ions can then be sorted based on their mass-charge-ratio with the help from electric and magnetic fields. It is possible to analyze solid, liquid and gas samples. The sample is ionized in the ion source, for example by being bombarded with electrons. Usually the sample is converted to cations. In the mass analyzer the ions are accelerated and depending on their mass and charge they are sorted and separated. This is done by electric or magnetic fields and ions with the same mass-to-charge ratio deflect in the same way. The

detector can measure charged particles and the ions are measured and presented on a chart. The chart is usually presented as a vertical bar graph where every bar represents ions with a specific mass-to-charge ratio. The longer the bar is, the higher is the relative abundance of the ion. Correlating the known masses to the identified masses or looking at characteristic fragmentation patterns it is possible to identify the atoms or molecules analyzed.

In paper I a specialization of mass spectrometry was used, isotope-ratio mass spectrometry. This method can measure the relative abundance of isotopes in a sample and in paper I it was used to measure $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio in breath samples. It was performed at the department of Clinical Chemistry by laboratory research technicians.

High-performance Liquid Chromatography

HPLC is a chromatographic method used to dissolve chemical compounds with a two-phase system, **Figure 10**. The sample is injected and first pumped through a pre-column cleaning the sample, increasing the life length of the analytical column. The sample then reaches the analytical column with a length of 10-500 mm and with an inner diameter of a few millimeters. The pump generates a high pressure in the column. Depending on the analytes of interest the analytical column contains particles with different polarity, often silicon particles covered with carbon chains with 8 or 18 carbon atoms. The dissolved analytes in the sample pass through the column and are either absorbed or adsorbed to the silicon particles. If the analytes have different polarities they will slow down in a different manner in the column and therefore have different time to pass through the column. The detector registers the different analytes, often with electro-chemic detection or light detection with infrared (IR) or ultra violet (UV).

HPLC was used in paper I-IV. In paper I levodopa in blood and SC tissue was analyzed and in paper II levodopa in blood and CSF was analyzed. In paper III-IV levodopa in blood and levodopa, DA, GABA and glutamate in the basal ganglia were analyzed. Analysis of levodopa in serum has previously been described (176, 177). In paper I and II the HPLC analyses were performed at the department of Clinical Chemistry by laboratory research technicians. In paper III and IV the HPLC analyses of the CNS dialysates were purchased from Pronexus Analytical AB, Stockholm, Sweden.

Figure 10. Schematic representation of a HPLC unit. 1. Solvent reservoirs, different depending on the compound to dissolve. 2. Solvent degasser removing air bubbles. 3. Gradient valve where the proportions between the different solvents are regulated. 4. Mixing vessel for delivery of the mobile phase. 5. High-pressure pump. 6. Switching valve in "inject position". 6' Switching valve in "load position". 7. Sample injection. 8. Pre-column (guard column). 9. Analytical column. 10. Detector. 11. Data acquisition. 12. Waste or fraction collector.

Deep Brain Stimulation

DBS as a treatment method in PD has been described in the Introduction section.

In paper III-IV STN DBS was performed. The DBS electrodes were implanted bilaterally in STN. Direct anatomical targeting of the STN, putamen and GPi was carried out on axial and coronal images recorded with 1.5-Tesla magnetic resonance imaging (MRI); (T1 and T2, slice thickness 2 mm; Philips Intera, Best, the Netherlands). The target points and trajectories for the DBS electrodes and the microdialysis probes were calculated using the Leksell Surgiplan (Elekta Instrument AB, Stockholm, Sweden). The microdialysis probes were fixed gently in the burr hole with soft bone wax and tunneled out through a separate skin incision 8–10 cm posterior on the skull. The microdialysis probes were manufactured with a small golden tip, making it visible on postoperative radiological follow up with computed tomography (CT). In order to further examine the sampling from the probes, patient specific models were set up where the maximum tissue volume of influence for each probe was simulated and visualized in relation to patient anatomy, **Figure 11**. The preoperative MRI and postoperative CT were fused to confirm the probe positions and visualize the patient anatomy. The simulations used the finite element method (FEM), which mathematically predicts and visualizes the distribution of a property (178), in this case the maximum tissue volume that is being sampled during brain

microdialysis. The simulations were based on tissue properties representing the average brain tissue and analyte properties representative for the neuroactive substances of interest. The simulation process and an *ex vivo* experiment have been described in detail by Diczfalussy *et al.* (179).

Figure 11. Illustration of an example of computational modelling with FEM, showing bilateral STN electrodes with the electric fields (green areas) and microdialysis probes with maximum tissue volume that is being sampled during brain microdialysis (brown areas).

Statistics

Paper I: Nonparametric test was used for the analysis due to the small number of subjects. Related-samples Wilcoxon signed-rank test was used to analyze differences in the levodopa concentrations in blood and SC tissue within group 2. Independent Mann-Whitney U test was used to analyze differences in the levodopa dose, levodopa concentrations and $T_{1/2}$ of GE between the two different PD groups and the reference group. Median values and box plot were also used to compare $T_{1/2}$ of GE. We also compared group differences for area under the curve (AUC) for levodopa levels in blood and SC tissue ($\mu\text{mol} \times \text{min/L}$). IBM SPSS Statistics 24 and Microsoft Excel were used for statistical analysis. P values below 0.05 were considered significant. Guidance from statistician was provided.

Paper II: A statistician helped with the choice of statistical methods and calculations. To calculate differences in C_{max} between the days ANOVA, with repeated measures, was used. Each patient was their own control when comparing the results between the 3 days. Linear trapezoidal summation to time was used to calculate the AUC and a 95% confidential interval (CI) was calculated for AUC. P values below 0.05 were considered significant.

Paper III: Different statistical methods were considered but due to small number of study subjects most results were presented as descriptive. Some median values were calculated using Microsoft Excel.

Paper IV: Because of data from only one patient the results were presented as descriptive. Mean values, C_{max} , minimum concentration (C_{min}) and differences in per cent were calculated using Microsoft Excel. Guidance from statistician was provided.

PATIENTS AND MATERIALS

Patient selection

Paper I: PD patients with either wearing off phenomenon (group 1; 16 patients; 7 men and 9 women) or with on-off syndrome (group 2; 14 patients; 7 men and 7 women), were included from the outpatient department. Mean age was 67.9 ± 7.5 and 65.0 ± 6.9 years for group 1 and group 2 respectively and mean disease duration of 10.1 and 12.1 years (range 6-16 years and 5-19 years respectively). The mean dose of oral treatment with levodopa was 800 mg (range 300-1700 mg) and 817 mg (range 400-2500 mg) for group 1 and group 2 respectively.

Paper II: PD patients with wearing off phenomenon and levodopa/DDI treatment but no COMT-inhibitor were included from the outpatient department. Five patients were included (2 men and 3 women) with the mean age of 68.9 ± 10.7 years and mean disease duration of 7.14 years (range 3.8-9.5 years). The mean dose of oral treatment with levodopa was 500 mg (range 400-650 mg).

Paper III: PD patients with advanced disease and dyskinesia that qualified for DBS surgery were included from the outpatient department. Five patients with advanced PD (mean age 56 ± 7 years) and disabling dyskinesia were included in the study. The first case was a woman and the following four were men. The mean disease duration was 10 years (range 8–16 years) and the mean dose of oral treatment with levodopa was 1140 mg (range 850–1300 mg).

Paper IV: Data from patient No. 4 in study III was presented. The patient was a 57-years-old man with PD since 8 years and with symptoms starting in the left side of the body. The patient had oral levodopa treatment for 7 years and suffered from dyskinesia. The daily levodopa dose was 850 mg in addition to rasagiline, entacapone and pramipexole.

Ethical approval was obtained for all studies (No. 97357, 20020115 and 51-04) and all the patients received written and oral information and gave their informed consent for participation.

Materials and Chemicals

Paper I: The microdialysis set was from CMA Microdialysis AB (Stockholm, Sweden) and consisted of a CMA/107 Microinjection Pump with one 2.5-mL CMA 106 syringe, which was connected to the IV probe. The IV probe was custom made at the time with a 20 mm membrane length (today available as CMA 64) and the CMA 60 probe was used for SC microdialysis. During breath test the patients breathed in 10 mL Vacutainer[®]. Chemicals used were Madopark[®] (100 mg levodopa + 25 mg benserazide), Roche AB, Sweden and Levodopa infusion 5 mg/mL (Levodopa, Fresenius Kabi AB, Uppsala, Sweden).

Paper II: The microdialysis set was from CMA Microdialysis AB (Stockholm, Sweden) and consisted of a CMA/107 Microinjection Pump with one 2.5-mL CMA 106 syringe, which was connected to the IV probe. The IV probe was custom made at the time with a 20 mm membrane length (today available as CMA 64). The samples were collected into vials containing 90 µL of a solution of hydrochloric acid 10 mmol/L and Na₂EDTA, 2 mmol/L. Lumbar drainage, Epidural Catheter 16G REF 100/382/116, Smiths, Hythe Kent (England). CADD-infusion set, SIMS Deltec, Inc, (St Paul, MN). Chemicals used were Carbocain[®] adrenalin, 5 mg/mL + 5 µg/mL, Astra Zeneca (Sweden), Levodopa IV infusion 5 mg/mL (Levodopa, Fresenius Kabi AB, Uppsala, Sweden), Comtess[®] (200 mg entacapone), Orion Pharma Espoo, Finland, and Lodosyn[®] (25 mg carbidopa), MSD, USA.

Paper III: The microdialysis set was from CMA Microdialysis AB (Stockholm, Sweden) and consisted of CMA/107 Microinjection Pumps with 2.5-mL syringes connected to both the brain (CMA 65) and the IV probes, respectively. Both microdialysis probes were manufactured by CMA Microdialysis AB. The length of the brain probe shaft was 190 mm and the length of the dialysis membrane was 10 mm, with a golden tip for radiologic confirmation of position. Chemicals used were Carbocain[®] adrenalin, 5 mg/mL + 5 µg/mL, Astra Zeneca (Sweden) and Levodopa IV infusion 5 mg/mL (Levodopa, Fresenius Kabi AB, Uppsala, Sweden).

Paper IV: Same as for paper III but with the addition of the chemicals Madopark[®] (100 mg levodopa + 25 mg benserazide), Roche AB, Sweden, Madopark[®] Quick mite (50 mg levodopa + 12,5 mg benserazide), Roche AB, Sweden and Comtess[®] (200 mg entacapone), Orion Pharma Espoo, Finland.

The dialysis membranes of all the probes in these studies consisted of a polyamide membrane with a molecular cut-off at 20 kDa (levodopa 197.19 Da, DA 153.18 Da) because of better recovery for catecholamines (64 %) compared to 100 kDa (13 %) and the dialysis solution consisted of Ringer Acetate. The use of 20 IU/mL of sodium dalteparin (Fragmin[®] from Pfizer, Sollentuna, Sweden) in the dialysis solution (Ringer Acetate[®], Braun, Germany) inhibited the formation of fibrin deposits on the membrane (147).

Levodopa from IV, SC and CSF samples and recovery was analyzed with a HPLC-system consisting of a P680 HPLC pump (Dionex, Germering, Germany), an automated sample injector ASI-100 (Dionex) and an electrochemical detector DECADE (Antec Leydon, Zoeterwoude, the Netherlands). The analytical column was an Aquasil C18 250 mm x 4.6 mm, particle size 5 μ m, with a preceding matched guard column Aquasil C18 10 mm x 4 mm x 5 μ m, both from Keystone Scientific (Keystone, CO, USA). The column temperature was set at 23°C with an integrated oven from Dionex. The mobile phase consisted of sodium 1-heptane-sulfonate 1 mmol/L, citric acid monohydrate 0.1 mol/L, Na₂-ethylenediaminetetraacetic acid 0.05 mmol/L and 5% Aceto nitrile, pH 2.7. Flow rate was set at 1.0 mL/min, the run time was set at 15 min and the detector was set at +750 mV (nA range) versus the Ag/AgCl reference electrode. Injection volume was 10 μ L. Chromatograms were measured using Chromeleon software from Dionex. All samples from the CNS were analyzed by Pronexus Analytical AB, Stockholm, Sweden.

REVIEW OF THE PAPERS AND MAIN RESULTS

Paper I.

Is levodopa pharmacokinetics in patients with Parkinson's disease depending on gastric emptying?

Oral levodopa is the gold standard in the treatment of PD. When given orally, levodopa has to pass through the upper GI tract before absorption in the small intestine. Levodopa is then transported in the systemic circulation to the brain where it can be converted to DA giving symptom relief to PD patients. As mentioned, GE is considered an important step for the availability of levodopa and GE has been shown to be delayed in many PD patients. It has been shown that CDS improves both motor complications and NMS in PD.

In paper I we wanted to study the GE rate in PD patients with motor complications (group 1 with wearing off phenomenon, group 2 with on-off syndrome) and to investigate if there is a correlation between GE and the levodopa uptake from the GI tract. We also wanted to see if CDS given as a continuous levodopa infusion IV for 10 days affected the levodopa uptake or the GE. All patients from both groups performed the protocol of Day 1.

Day 1: A microdialysis probe was placed in a brachial vein and another probe was placed in the abdominal SC tissue. Baseline monitoring for microdialysis and breath test was performed before intake of an omelet containing a standardized amount of protein and ^{13}C marked octanoic acid. Together with the omelet the patients were given 1 tablet of Madopark[®] (100 mg of levodopa/25 mg of benserazide). During a period of 4 h IV dialysates and breath samples were taken every 15-min and SC dialysate fractions were collected every 30-min.

Day 2 - 12: The patients from group 2 were also treated with continuous IV levodopa infusion, 5 mg/mL during 12 h daily for 10 days. The daily dose of levodopa infusion was calculated to correlate to the patients' oral medication. After the infusion period the patients in group 2 underwent the same procedure as on Day 1.

In paper I we could see significantly higher levels of levodopa in PD patients with wearing off phenomenon compared to patients with on-off syndrome, **Figure 12.**

Figure 12. Levodopa concentrations (mean values) in blood and SC tissue from PD patients with wearing off phenomenon (group 1) and with on-off syndrome (group 2) before and after 10 days of levodopa infusion treatment. IV and SC sampling were performed at 15-min and at 30-min intervals respectively.

The GE was significantly delayed in PD patients compared to the reference group but there was no difference between the two PD groups, **Figure 13**.

Figure 13. Boxplot for T_{1/2} of GE with significance levels (p values) for reference group, PD patients with wearing off phenomenon, PD patients with on-off syndrome before and after 10 days of levodopa infusion treatment and the two PD groups fused. Horizontal line in box=median value. Lower part of box=first quartile. Upper part of box=last quartile. Vertical lines from box=smallest and largest non-outliers. Outliers (*) are also presented.

We found no obvious relation between GE and levodopa uptake and CDS did not affect GE but it resulted in significantly decreased levodopa levels.

Paper II.

The effect of peripheral enzyme inhibitors on levodopa concentrations in blood and CSF

Without enzyme inhibitors most of the orally given levodopa is metabolized by AADC and COMT before reaching the brain. Therefore levodopa is always given together with a peripheral DDI such as Madopark® (levodopa + benserazide) or Sinemet® (levodopa + carbidopa) and it is also possible to add a peripheral COMT-inhibitor such as entacapone. This increases the bioavailability of levodopa considerably and more levodopa can pass the BBB and be converted to DA in the brain. It has been shown that increased $C_{\max}$ might be an important factor in developing motor complications in PD patients by inducing modifications of postsynaptic dopaminergic receptors in brain. However, previous studies have shown that $C_{\max}$ in blood is unaltered when adding the COMT-inhibitor entacapone to levodopa/DDI and it was believed that this also accounts for $C_{\max}$ of levodopa in the brain. This is considered of importance in avoiding motor complications in PD.

In paper II we wanted to study the levodopa levels in blood in PD patients with wearing off phenomenon and to investigate how much of levodopa from blood crosses the BBB by comparing the levodopa levels in CSF. We also wanted to study the effect of the COMT-inhibitor entacapone and the DDI carbidopa on levodopa levels in blood and CSF.

PD patients with levodopa/DDI treatment and wearing off phenomenon were included and all patients underwent microdialysis in blood and sampling of CSF during the following treatments:

Day 1: Levodopa 30 mg/h was given IV during a 12-h period.

Day 2: Levodopa 30 mg/h was given IV during a 12-h period. Oral entacapone, 200 mg, was added and the first dose was taken immediately after the collection of the baseline samples, the second after 4 h and the third after 8 h.

Day 3: Levodopa 30 mg/h was given IV during a 12-h period. The same adding of entacapone was made with an addition of oral carbidopa, 25 mg, at 4 and 8 h, respectively after the first entacapone dose. The first dose of carbidopa was given at 4 h after the first entacapone dose for showing that the levodopa/entacapone dose at day 3 gave levodopa concentrations at the same level as at day 2.

In paper II we could see a significant increase of levodopa $C_{\max}$ (mean difference) in both CSF and blood (11% and 33 % respectively) when adding entacapone and when adding entacapone + carbidopa (121 % and 183 % respectively) compared to levodopa given alone, **Table 1**. When adding entacapone we could show an increase of mean levodopa AUC in CSF and blood (12% and 38 % respectively) and also when adding entacapone + carbidopa (50 % and 73 % respectively) compared to levodopa given alone, **Table 2**. Thus the increase of $C_{\max}$ and AUC was most evident when both entacapone and carbidopa were added together.

Table 1. Comparison of mean differences of levodopa $C_{\max}$ in CSF and blood.

	Comparison in $C_{\max}$		
	Day 1–Day 2	Day 2–Day 3	Day 1–Day 3
Mean difference in $C_{\max}$, CSF	1.11 (11%) $P = 0.032$	2.04 (104%) $P < 0.001$	2.21 (121%) $P < 0.001$
Mean difference in $C_{\max}$, blood	1.33 (33%) $P < 0.001$	1.83 (83%) $P < 0.001$	2.83 (183%) $P < 0.001$

Table 2. Comparison of mean levodopa AUC in CSF and blood.

	AUC ($\mu\text{mol L}^{-1} \text{h}$)		
	Day 1	Day 2	Day 3
Mean AUC, CSF	3.4 95% CI: 0.39–6.4	3.8 95% CI: 0.04–7.6	5.1 95% CI: 0*–12.3
Mean AUC, blood	27.6 95% CI: 0*–58.9	38.0 95% CI: 16.7–59.4	47.8 95% CI: 0*–110.3

*Negative value in the calculation

The levodopa levels in CSF were 12.3 % of those in blood when comparing mean AUC without enzyme inhibitors (day 1) and this is comparable to previous data (40). During sampling time the levodopa levels in blood reached a plateau all 3 days. The plateau was almost reached in CSF when entacapone was added (day 2), but could not be seen when the addition with entacapone + carbidopa was made (day 3), **Figure 14**.

Figure 14. The mean levodopa concentrations in CSF and blood during treatment with continuous IV levodopa infusion 30 mg/h alone and with concomitant treatment with oral entacapone and carbidopa.

Paper III.

Neurotransmitter levels in basal ganglia during levodopa and deep brain stimulation treatment in Parkinson's disease

The amount of studies where neurotransmitters in brain are studied in human *in vivo* are limited, both because of ethical considerations and because of the delicate structures involved. However, it is of importance for better understanding of the mechanisms underlying PD and during treatment of PD patients. STN DBS has been proven a successful treatment in PD when the patients suffer from motor complications. The exact mechanisms behind STN DBS are still unclear and different theories are presented in the "Deep Brain Stimulation" section under "Treatment of Parkinson's Disease".

In paper III we wanted to study the effect of STN DBS on the levels of different neurotransmitters, for example DA, in right putamen, right Gpi and left Gpi in PD patients with disabling dyskinesia. We also wanted to see how levodopa given as an IV infusion affects these neurotransmitter levels and also the effect on levodopa levels in brain during STN DBS and levodopa infusion. The study design was as follows:

Day 1: The patients were admitted to the ward and their antiparkinsonian medication was discontinued the night before DBS surgery.

Day 2: The DBS electrodes were implanted bilaterally in STN. During the surgery three microdialysis probes were implanted, one each in the right putamen and bilaterally into the Gpi. The probes were connected to the pumps immediately after implantation and flow rate was set at 0.5 $\mu\text{L}/\text{min}$. Microdialysate sampling started at 6 pm, app. 3 hours after DBS surgery and continued during the entire study period, until Day 5 at 8 am. Probe and tubing delayed the fraction samples with app. 6 min. Fractions were collected every hour during daytime and every two hours during night (9 pm – 8 am).

Day 3-4: STN DBS was performed according to a specific protocol.

Day 4: A microdialysis probe was placed in a brachial vein and samples were collected every hour. IV levodopa infusion (75 mg/h) was given in the contralateral arm during 3 h. After one hour baseline recording, levodopa infusion combined with bilateral STN DBS was performed for another 6 h. The IV microdialysis was then discontinued.

Day 5: The last CNS microdialysis samples were taken at 8 am and all the probes were then removed.

In this paper we could see that the DA levels in right putamen increased during STN DBS, **Figure 15**.

Figure 15. The mean DA concentrations in the right putamen from patients 2–5. Left-sided STN DBS (A–B) induced a 204% increase of DA. New baseline fractions (B–C) were collected with no STN DBS. During right-sided STN DBS (C–D), the concentrations continued to decrease another 25%, as in the baseline fractions after the left-sided stimulation. The total decrease was 40% including the baseline fractions after the right-sided stimulation (D–E). During bilateral stimulation (E–F), DA increased 35%, and after the DBS period the concentration returned to basal levels (F–G). During IV levodopa infusion (G–H), the DA concentrations increased markedly. The concentrations decreased rapidly when the infusion was stopped during the baseline fraction (H–I). Concomitant bilateral STN DBS with levodopa infusion increased DA concentrations to higher levels than the infusion alone (I–J). J and onward shows bilateral STN DBS alone.

In addition, an increase of GABA concentrations in the Gpi during STN DBS and during levodopa infusion was found, **Figure 16**.

Figure 16. The mean GABA concentrations in the left Gpi, from patients 2–5, during IV levodopa infusion (G–H), baseline (H–I), IV levodopa infusion with concomitant bilateral STN DBS (I–J) and during bilateral STN DBS alone (J and onward).

We could also see that STN DBS seemed to interfere with levodopa therapy resulting in higher levels of levodopa in the brain, **Table 3**.

Table 3. Mean levodopa concentrations in globus pallidus interna (Gpi) and right putamen (Put) compared to blood for all patients.

	Gpi/blood %	Put/blood %
Basal value	10.1	5.8
L-dopa infusion 75 mg/h	18.6	16.8
L-dopa infusion 75 mg/h + STN DBS	20.3	19.4
L-dopa, levodopa; STN DBS, subthalamic nucleus deep brain stimulation.		

Paper IV.

Levodopa pharmacokinetics in brain after both oral and intravenous levodopa in one patient with advanced Parkinson's disease

During study III patient 4 received oral levodopa treatment not according to protocol but due to distressing parkinsonism on several occasions following STN DBS surgery but before the start of the stimulation period. The oral medication was not discovered until after the dialysates were analyzed and the patient then approved to publish the data. Microdialysis sampling had started before the levodopa intakes and therefore we had the opportunity to investigate the effects on levodopa and DA levels in brain during oral levodopa treatment in this PD patient. As far as we are aware, there is no *in vivo* study on this in humans.

In paper IV the levodopa and DA levels in one patient with PD for 8 years and disabling dyskinesia were investigated. The study design was as in paper III but the patient was given IV levodopa infusion for 1 + 7 hours (instead of 3 + 6 h) due to technical failure in the beginning of the infusion period and prolonging of the second infusion period. The patient received oral PD medication on 5 occasions during a 10-h period, **Figure 17**.

Tablet intake

Madopark Quick Mite 50/12,5 mg = MQM 50
 Madopark Quick Mite 25/6,25 mg = MQM 25
 Madopark Depot 100/25 mg = MD 100
 Comtess 200 mg = C 200

Figure 17. Time table for oral levodopa intake and STN DBS surgery. MQM 50=Levodopa/benserazide, 50/12.5 mg; MQM 25=Levodopa/benserazide, 25/6.25 mg; MD 100=Levodopa/benserazide, 100/25 mg sustained release; C 200=Entacapone 200 mg.

In this paper we could see that levodopa, both given orally and IV in this PD patient, resulted in increased levels of levodopa and DA in the BG. During oral medication levodopa and DA levels followed each other, **Figure 18**. We could also see that STN DBS seemed to increase DA and that right stimulation seemed to have a more pronounced effect on right structures in striatum (right putamen and right Gpi) while left stimulation seemed to have a more pronounced effect on left structures (left Gpi).

Figure 18. The mean concentrations of levodopa and DA in the BG during PD medication intake. Arrow=fraction with PD medication intake.

DISCUSSION

In these four papers, we can follow levodopa from stomach to brain. First, we follow the passage of levodopa through the upper GI tract investigating the GE and its correlation to levodopa uptake in paper I. Levodopa is then transported over the intestinal mucosa to the peripheral circulation and then transported over the BBB to the brain and in paper II we investigate the effects of enzyme inhibitors on the levodopa levels in blood and in CSF. The levodopa is converted to DA in the brain and in paper III-IV the levels of, for example levodopa and DA are studied when PD patients receive levodopa treatment, with and without STN DBS.

We could see a more favorable levodopa uptake in PD patients earlier in disease indicating that PD patients with less severe disease have a better uptake of levodopa from the intestine. However, there was no significant difference in GE between PD patients with severe and less severe disease, indicating that other factors than GE influence the levodopa uptake from the GI tract. It has earlier been shown that the absorption of levodopa from the gut is accelerated after long exposure to levodopa (180, 181) and that age also seems to affect levodopa uptake since higher levels of levodopa has been seen in older PD patients (49, 182). However, in our study neither significant age difference nor any obvious time difference of levodopa treatment was seen in the two PD groups.

There was a significant delay of GE in the PD groups compared to the reference group supporting the theory of delayed GE in PD in complication phase. There is no conclusive data about the relation between PD stage and GE (25, 27, 28, 31, 143-145) and it is possible that there is no strict relation between severity of motor symptoms and degree of impairment of GE in PD patients. This is supported by our results where we could see no difference of GE between the two PD groups. One hypothesis is that PD pathology starts in the gut and spreads like a prion-like disease towards the CNS. It is possible that mechanisms for GE are affected by these changes and that they appear early in disease, probably even before motor symptoms and PD diagnosis, and therefore there might be no obvious difference of GE in PD patients with different stages of disease.

A 10-day infusion period with levodopa IV in the PD group with severe on-off, resulted in significantly lower levodopa concentrations indicating an effect caused by CDS. Previous studies have shown that CDS seems to induce plasticity changes of the dopaminergic postsynaptic receptors in brain reducing motor complications (20, 111, 112). We wanted to study if CDS in this way also could induce changes in the GI tract affecting the levodopa uptake. A question that remains to be answered is why the levodopa levels decreased after CDS and did not increase. However, the levodopa curves, both in blood and in SC tissue,

seemed smoother after CDS, **Figure 12**. This decrease of fluctuations in levodopa might be the positive effect of CDS, possibly retarding further development of motor complications. We could not see any difference of GE after the infusion period which suggests that the mechanisms of GE are not influenced by CDS. More likely the GE is regulated locally in the GI tract. However, **Figure 13** indicates more gathered results of GE after infusion treatment and possibly there is an effect of CDS on GE which the small patient number in this study failed to show.

Additional entacapone to levodopa therapy significantly increases the $C_{\max}$ and AUC of levodopa in both blood and CSF and the increase is more evident when entacapone is combined with carbidopa. Several previous studies have stated that entacapone increases the levodopa AUC without affecting the $C_{\max}$ when added to levodopa/DDI (62, 67, 68) and this has been considered important in delaying the development of motor complications in PD patients. Our results in paper II show that the levodopa $C_{\max}$ indeed is increased both in blood and in CNS when adding entacapone to levodopa and that the effect is even more pronounced when adding both carbidopa and entacapone to levodopa. There are some possible explanations why our results concerning levodopa $C_{\max}$ differ from previous studies. For example, entacapone was only given as a single-dose together with levodopa/DDI in some of these studies (60, 62), while in our study entacapone was given at 4-h intervals, more similar to the clinical situation for PD patients treated with additional entacapone. In their study, Kaakkola *et al.* gave additional entacapone to each dose of levodopa, but blood samples were only taken after the first morning dose (59), while we collected samples for 12 h. Our results showed an increase of levodopa $C_{\max}$ in both CSF and in blood, and this is important to take into consideration when starting additional treatment with entacapone to levodopa/DDI. In the STRIDE-PD study, adding entacapone to levodopa/DDI early in disease was shown to induce a higher frequency of dyskinesia earlier in treatment compared to levodopa/DDI (183). However, no reduction of levodopa/DDI dose was made. The results from the STRIDE-PD study are in accordance with our findings in paper II and imply that reduction of the levodopa/DDI dose is of importance, when adding entacapone, to avoid high levodopa peaks that could accelerate the neuronal changes and the development of motor complications.

In paper II the levodopa levels in blood reached a plateau all three days. In CSF plateau levels were almost reached when entacapone was added alone to levodopa but could not be seen when both entacapone and carbidopa were added, **Figure 14**. A longer sampling time than the 12 h/day we had would have been preferable for evaluating the true effect of the enzyme inhibitors on levodopa $C_{\max}$. However, this would have been more uncomfortable for the patients. It would also have been preferable to make an addition of carbidopa alone to levodopa. However, this would have prolonged the study with an additional day,

possibly resulting in more discomfort for the patients and an increased risk of infection with the lumbar catheter.

A previous study has shown delay for levodopa concentrations in brain tissue compared to blood (130). In paper I we could see that the levodopa curves in blood and SC tissue had very similar appearances but with a slight delay in the SC tissue. These results are similar to previous findings (147) and suggest that the levodopa pharmacokinetics is more similar in SC tissue and brain tissue compared to blood and brain tissue. This might be explained by the fact that brain tissue is more lipophilic and therefore more alike SC tissue. Monitoring of SC levodopa levels might therefore be more appropriate than blood concentration monitoring for clinical studies concerning correlation between levodopa levels and patient mobility.

During STN DBS and during levodopa infusion the DA levels in the right putamen and the GABA levels in the Gpi increased. These findings indicate that the STN has a direct action on the SNc and that STN DBS might indirectly release putaminal DA, **Figure 19**.

Figure 19. The basal ganglia network. A. Normal state. B. Parkinson's disease. C. Parkinson's disease during levodopa infusion with concomitant bilateral STN DBS. **Direct pathway:** DA release from SNc activates D1 receptors and Gpi/SNr is then inhibited. This results in less inhibition of the thalamus which in turn results in activation of cortex. **Indirect pathway:** DA release from SNc activates D2 receptors and Gpe is then inhibited. This results in less inhibition of STN which has an activating effect on Gpi/SNr and therefore thalamus is inhibited resulting in less activation of cortex. Red arrow=inhibitory, green arrow=excitatory, grey arrow=DA transmission, black arrow=other neuronal networks, VA=ventral anterior nucleus, VL=ventral lateral nucleus, D1=D1-like DA receptors, D2=D2-like DA receptor, Gpe=Globus pallidus externa, Gpi=Globus pallidus interna, SNc=Substantia nigra pars compacta, SNr=Substantia nigra pars reticulata, STN=Subthalamic nucleus, PPN=Pedunculopontine nucleus.

There is also evidence that STN DBS interferes with levodopa therapy resulting in higher levels of levodopa in the brain. It has been shown that it is possible to decrease levodopa medication after STN DBS surgery (123-127) and our results might give one explanation for that.

Levodopa levels increased promptly in brain after both oral and IV levodopa medication and it seemed to be quickly metabolized to DA since the levels of DA correlated well with the levodopa concentrations, but with a slight delay, **Figure 18**. It also seemed as if levodopa was accumulated extracellularly in brain and this could indicate a longer $T_{1/2}$ of levodopa in brain compared to peripheral blood. Both the DDI benserazide and the COMT-inhibitor entacapone were given during the period of oral medication and adding a DDI or entacapone to levodopa have previously been shown to increase the $T_{1/2}$ of levodopa in blood (35, 58-62). Adding the COMT-inhibitor entacapone to levodopa/DDI also results in higher levels of levodopa in brain (66). The possible extracellular accumulation of levodopa could indicate difficulties for the DA producing neurons to handle all levodopa available, the neurons could be saturated with levodopa. Our results indicate that PD patients with advanced disease still have capacity to metabolize levodopa to DA despite probable pronounced nigral degeneration and the question is if other structures/neurons than the dopaminergic neurons are involved in the metabolism of levodopa to DA. Serotonergic neurons are able to convert exogenous levodopa to DA and release it as a “false transmitter” giving symptom relief in PD patients (71-75) and may therefore play a role in the converting process of exogenous levodopa to DA. One theory is that the autoregulating function of the DA release is lacking in serotonergic neurons resulting in an un-controlled DA release after levodopa administration and thus causing LID (90-92). The serotonergic hyperinnervation and the dysregulated DA release in different areas of the brain could also be possible actors in the origination of some NMS in PD, for example impaired cognition, depression and anxiety (8, 93-100).

As mentioned earlier it seems to be of importance to avoid high levodopa peaks in brain in PD patients and that CDS has been shown effective in reducing LID. In paper IV we could see a more discrete difference between C_{max} and C_{min} of levodopa during the levodopa infusion compared to the oral medication indicating less pronounced levodopa peaks in brain during levodopa infusion. Levodopa infusion might therefore be more favorable than oral levodopa treatment in delaying the development of LID in PD patients.

In paper IV we could also see that STN DBS seemed to induce an increase of DA in the left-side structures during left-side stimulation and an increase of DA in the right-side structures during right-side stimulation indicating a direct action on SNc as we also found evidence for in paper III. This supports the theory that DBS activate fiber tracts surrounding the stimulated area.

CONCLUSIONS

Concluding the results in paper I-IV we could see;

- A more favorable levodopa uptake in PD patients with wearing off phenomenon compared to PD patients with on-off syndrome indicating a better levodopa uptake earlier in the disease. This might in part explain why higher doses of levodopa are needed as the disease progress and the increased doses might accelerate the development of motor complications. It might therefore be of importance to consider more continuously administrated PD medication in an attempt to lower the levodopa dose and to obtain smoother levodopa levels in blood and in brain.
- A delayed GE in PD patients but no difference between the two PD groups. This indicates that there is no obvious relation between levodopa uptake and GE or PD stage and GE in PD patients with motor complications. It is also possible that the delayed GE in PD patients appear already in the asymptomatic phase of the disease.
- That CDS resulted in lower levodopa levels in both blood and SC tissue but it did not affect the GE indicating that CDS has no effect on the mechanisms of GE but on the mechanisms of levodopa uptake. The possibly smoother levodopa curves after CDS might delay further development of motor complications.
- That the $C_{\max}$ and AUC of levodopa in both CSF and blood increased significantly when additional entacapone was given alone and in combination with carbidopa. The increase was more evident when entacapone was combined with carbidopa. This study is, to our knowledge, the first to show that levodopa $C_{\max}$ indeed is increased in CNS when the peripheral COMT-inhibitor entacapone is added to levodopa/DDI.
- That it is important to take into account the increased $C_{\max}$ and therefore consider a decreased levodopa dose when adding entacapone to levodopa/DDI. It is possible that the same applies for other COMT-inhibitors than entacapone and the effect might be even more pronounced with more effective preparations.
- That STN DBS increases DA in the right putamen and increases GABA in the Gpi. This indicates a direct action on the SNc by STN DBS and that STN DBS might indirectly release putaminal DA and result in a decreased suppression of the thalamocortical loop, **Figure 19**.

- That STN DBS seems to interfere with levodopa therapy resulting in higher levels of levodopa in the brain. This might explain why it is possible to decrease levodopa medication after STN DBS surgery.
- That PD patients with advanced disease and probable pronounced nigral degeneration still seem to have the capacity to metabolize levodopa to DA in brain. The question is if the converting process occurs in remaining dopaminergic neurons, in other neurons or in other structures in brain.

Writing this doctoral thesis has awakened new thoughts about possible future projects. For example to study intermittent versus continuous levodopa treatment and to investigate the effects of these treatments at cellular level in brain *in vitro* and trying to find support for this *in vivo*. It would also be interesting to further study the effects of STN DBS on levodopa to gain more knowledge about the mechanisms behind the ability to decrease the levodopa dose after STN DBS surgery. In this thesis we could see that PD patients with advanced disease and probable pronounced nigral degeneration still seem to have the capacity to metabolize levodopa to DA and to investigate in what structures levodopa metabolism takes place in advanced PD would also be very interesting. However, the research of PD will continue to result in better treatments for the PD patients and hopefully eventually there will be a cure for this disease.

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