

COEXISTENCE OF NEUROTRANSMITTERS IN PARKINSON'S DISEASE

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ABSTRACT

Parkinson's disease is a neurological disorder of older individuals frequently encountered in clinical practice. Disparate etiologic factors including infectious agents, genetic predisposition, environmental and toxic factors, metabolic abnormalities, traumatic events, electromagnetic radiation exposure, antioxidants, sex hormones, pharmaceutical drugs and others have been proposed. In this study, cerebrospinal fluid obtained by lumbar puncture is used to assess levels of neurotransmitters in Parkinson's disease patients treated with L-dopa during On and Off periods. We found a correlation between small peptides such as neuropeptide Y, substance P and cholecystokinin (both octa- and tetra- peptides) with the classical neurotransmitters such as norepinephrine, dopamine and serotonin.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder of the central nervous system (CNS) that occurs most commonly in elderly people - about 1% of the population over the age of 65 is affected.¹ Symptoms result from a single transmitter deficit due to the loss of dopamine in the substantia nigra, pars compacta. The etiology is unknown, but epidemiological studies have suggested that exposure to toxins and viruses may confer a predisposition, along with life events and the aging process. It is likely that about 70-80% of nigrostriatal neurons have to cease functioning or die before symptoms appear.² Although nigral dopamine depletion is the dominant deficit, other monoamine transmitters, such as the noradrenergic locus coeruleus neurons,^{3,4,5} as well as other central and peripheral systems such as serotonergic, cholinergic and peptidergic pathways are also affected.^{6,7}

Although genetics likely plays a role in PD, most researchers believe environmental exposures increase a person's risk of developing the disease. There are a number of toxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, or MPTP (found in certain forms of synthetic heroin), that can cause Parkinsonian symptoms in humans. Other, still-unidentified environmental factors

also may cause PD in genetically susceptible individuals. Several lines of research suggest that mitochondria may play a role in the development of PD. Mitochondria are the energy-producing components of the cell and are major sources of free radicals molecules that damage membranes, proteins, DNA, and other parts of the cell.^{7,8} The importance of decreased efficiency of mitochondrial oxidative phosphorylation activity is multi-factorial. Perhaps the most important consequence of inefficient energy production is a change in the neuronal transmembrane potential. Several factors such as inhibition of mitochondrial respiration, generation of hydroxyl and nitric oxide radicals and reduced free radical defense mechanisms causing oxidative stress have been postulated to contribute to the degeneration of dopaminergic neurons.⁸⁻¹⁰

On the other hand, the discovery of several genetic mutations associated with PD raises the possibility that these or other biomarkers may help to identify persons at risk of PD.¹⁰ Some researchers even speculate that the protein buildup is part of an unsuccessful attempt to protect the cell. While mitochondrial dysfunction, oxidative stress, inflammation, and many other cellular processes may contribute to PD, the actual cause of dopamine cell death remains undetermined.¹¹

The most effective therapy is L-dopa (levodopa), which is most successful during the first years of PD. However, after 5-10 years of L-dopa replacement, 80% of PD patients show on and off phenomena with dyskinesias.¹¹ This is most notable in advanced stages, where the medication is unable to improve motor and non-motor parkinsonian features and does not halt PD progression. Levodopa can cross the blood-brain barrier and reduces morbidity and mortality in PD. Despite disadvantages, it remains the gold standard for the treatment of PD, mainly on account of impressive efficacy on motor symptoms and inexpensive price. Several motor features typically do not respond to levodopa. Speech, gait, posture and balance tend to deteriorate over the time. Side effects include hallucinations, cognitive impairment and orthostatic hypotension.

Neuropeptides, among other neurotransmitters, are widely distributed throughout the brain in specific nerve cells, coexisting with monoamines and/or other neuropeptides.^{12,13} They are believed to participate in several physiological and patho-physiological processes, including pain sensation, memory, neuroendocrine functions, regulation of release of monoamine transmitters and regulation of mood.¹⁴ The effects are brought about by primary actions of neuropeptides or their modulation of the effects of monoamine transmitters. Several peptides such as neuropeptide Y (NPY), cholecystokinin (CCK), substance P (SP), somatostatin and vasopressin are known to co-localize with classical neurotransmitters within a single neuron, which provides a means to transmit more complex types of signals.¹⁵ On the basis of co-existence with monoamine transmitters and distinct regional distribution, the assumption has been made that neuropeptides play a role in CNS disorders.¹⁶

This study deals with CSF analysis of small peptides such as substance P, neuropeptide Y, both the tetra- and octapeptides of cholecystokinin, and the correlation between catecholamines and small peptides, as well as catecholamines and their metabolites in both on and off PD patients. These levels are reported with the assumption that CSF concentration reflects brain or spinal cord concentrations and perhaps synaptic activity, since CSF is in constant exchange with the extra-cellular fluid of the CNS. Based on the fact that most of the transmitters in the brain are present in the CSF and that alterations in the concentration of transmitters in the CSF can alter the same transmitters in the brain,¹⁷ the results are compiled in PD patients (On and Off groups) and compared with healthy subjects.

METHODS

A total of 36 patients (15 women and 21 men) with PD were recruited from the Department of Neurology, Huddinge University Hospital, Stockholm, Sweden. The Ethics Committee of the Karolinska Institute, Stockholm, approved the study and all patients gave written informed consent to participate.

Patients were divided into two groups. The first group included 18 patients, each at different stages of illness (Hoehn and Yar range 2-4), who were on L-dopa dose 250 mg per day and had taken it in combination with other drugs with positive response towards their motor activities (PD On group). Another 18 PD patients who had been treated for 6-8 years with similar drug therapy, and showed motor fluctuations with severe off response (PD Off group) were also included. Both On and Off groups were monitored one week before samples of blood and CSF were taken. Food and fluid intake were similar in both groups and exact dose timings were maintained. Blood and CSF were taken early in the morning, between 6-8 AM, in a fasting condition. A control group of 18 healthy volunteers (recruited from among the Karolinska Institute employees) were used for comparison, and had their blood and CSF samples collected under conditions similar to the patient groups. None of the healthy controls had been on any medication for the past six months. For both PD cases and healthy controls, 10-12 ml CSF was collected in the sitting position at the L4-L5 levels. Blood samples were collected by venipuncture. Serum and CSF albumin and IgG levels were determined using Hitachi 737 Automatic Analyzer (Naka Works, Hitachi Ltd., Tokyo, Japan). CSF and serum samples were kept at -80°C if not analyzed immediately.

Concentrations of dopamine (DA), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), noradrenaline (NA), 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were measured using previously described methods.¹⁸ Measurements were also made in serum, but since no differences were found these were not considered further. Group analysis was done using ANOVA and comparisons were made via t-test. A p-value of <0.05 was considered significant.

RESULTS

Baseline data on both PD groups along with healthy controls are shown in Table 1.

Table 1: Clinical data on the healthy controls and PD Patients in both On and Off groups

Patients	N	Females	Age (years)	CSF-Albumin	CSF-IgG	IgG-index
Controls	18	7	53 ± 7	218 ± 17	34 ± 4	0.44 ± 0.01
PD (On)	18	8	72 ± 12	332 ± 42**	51 ± 7**	0.43 ± 0.01
PD (On)	18	9	74 ± 14	313 ± 37*	44 ± 6*	0.42 ± 0.01

*p < 0.05, ** p < 0.01

In figure 1, we present CSF levels of dopamine and its metabolites in PD patients and healthy controls. Both Dopamine (DA) and its metabolite homovanillic acid (HVA) decreased significantly in both On and Off PD patients, while the level of 3,4-dihydroxyphenyl acetic acid (DOPAC) remained unchanged. Figure 2 shows CSF levels of noradrenaline (NA) and its metabolites 3-methoxy-4-hydroxy phenyl glycol (MHPG) in PD patients and healthy controls. Figure 3 shows the correlation between NA and NPY in CSF of PD patients and healthy controls. Figure 4 shows the correlation between dopamine and CCK octapeptide in CSF of PD patients and healthy controls. Figure 5 shows the correlation between NA and CCK tetrapeptide in CSF of PD patients and healthy controls. Figure 6 shows the correlation between 5-HT and substance P in CSF of PD patients and healthy controls.

DISCUSSION

Parkinson's disease is an inexorably progressive disorder that worsens over time. The rate of nigral cell death is not exactly known, but neuro-imaging techniques estimate cell death occurs at a rate of approximately 10% per year.¹⁹ PD symptoms are considered to be a consequence of an imbalance between stimulatory and inhibitory impulses in the extrapyramidal system, and that is mainly due to DA transmission in the nigrostriatal pathway.

Studies suggest that PD can develop in populations exposed to pesticide, herbicide, well water and rural living, and that the disease is not dependent on cigarette smoking and caffeine consumption.²⁰ The diagnosis of PD generally relies on clinical observation of the combination of four cardinal motor signs, namely tremor, rigidity, bradykinesia and balance impairment or postural instability.²¹ These symptoms, especially the first three, are typically improved by dopamine replacement therapies, and a positive response to L-dopa is mandatory for the diagnosis of idiopathic PD. Long-term use of carbidopa-levodopa can cause some potentially disabling complications, including dyskinesias (involuntary muscle movements or jerky motions), blepharospasm (involuntary

contraction of eyelid muscle), motor fluctuations, and hallucinations.²² Motor fluctuations involve random conversion from a mobile state to an immobile state and vice versa, also known as the "on-off" phenomenon.²³ They are associated with fluctuating responses to L-dopa. Patients respond to the drug during the "on" state and are unresponsive during the "off" state. Most PD patients treated with L-dopa develop fluctuations in motor performance. After 3-5 years of treatment one-third, after 5-7 years about half, and after 10-12 years, nearly all patients suffer from motor fluctuations. Our results suggest that neurotransmitter modulations,

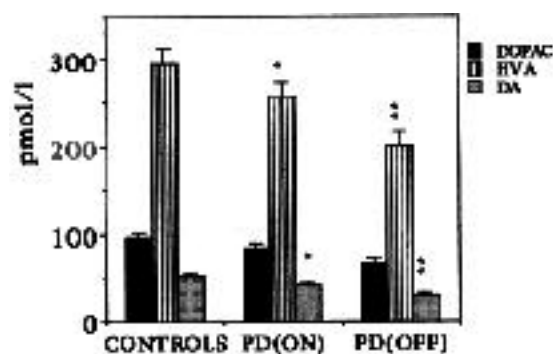


Fig. 1: CSF levels of dopamine (DA) and its metabolites, HVA and DOPAC in Parkinson patients (On and Off status) and healthy subjects

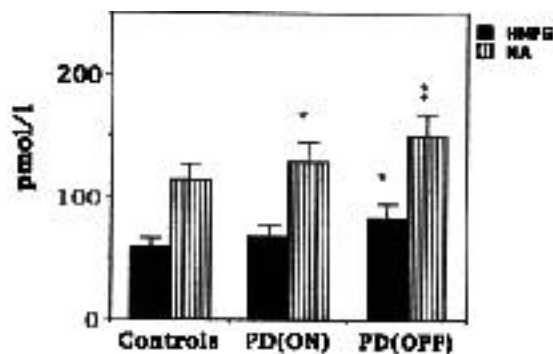


Fig. 2: CSF levels of norepinephrine (NA) and its metabolite, HMPG in Parkinson patients (On and Off status) and healthy subjects

possibly in tandem with neuropeptide effects, may be the mechanistic explanation underlying motor fluctuations in PD.

An unbalanced overproduction of reactive oxygen species (ROS) may give rise to oxidative stress which can induce neuronal damage, ultimately leading to neuronal death by apoptosis or necrosis. The clinical evidence demonstrating that antioxidant compounds can act as protective drugs in neurodegenerative disease is, however, relatively scarce. The major challenges for drug development are the slow kinetics of disease progression, the unsolved mechanistic questions concerning the final causalities of cell death, the necessity to attain an effective permeation of the blood-brain barrier, and the need to reduce the high concentrations currently required for protective effects in cellular and animal model systems.

The main metabolites of DA are HVA and DOPAC, and of NA, 3-methoxy-4-hydroxyphenylglycol (MHPG). In PD patients, the CSF NA and its metabolite, MHPG are increased in both on and off groups while DA and its metabolites DOPAC and HVA are significantly decreased in both groups. Figures 1 and 2 show CSF levels of DA, NA, and their metabolites in on and off PD patients, and are compared with healthy controls. A single dose of L-dopa gives a consistent response to motor activity in on situation whereas when the disease progresses within a few years, the response duration gets shorter, which is when DA and its metabolites are further decreased in the off situation (narrowed therapeutic window).

Apart from catecholamines, the estimation of serotonin (5-HT) is related to depression, and it plays a very important role in the CNS. We have already reported that levels of tryptophan, 5-HT and 5-HIAA are significantly decreased in both groups as both these groups are known to be depressed and inactive.²⁶ Apart from small neurotransmitters such as catecholamines and amino acids, recent research has also pointed to the role of peptides in CNS.²⁴⁻²⁶

One of the major reasons for analyzing CSF peptides of plasma and serum is that neuropeptides do not readily cross the blood-brain barrier.¹⁰ Changes in the CSF peptide concentration might be attributed to certain symptoms and can even be interpreted as characteristic features of certain pathological conditions. Neuropeptide Y, cholecystinin and substance P are some of the important peptides that are believed to play vital biological roles in various degenerative disorders.

Among the neuropeptides, NPY plays an important role in anxiety, depression and eating disorders. The coexistence

of NPY with other neurotransmitters and its wide distribution in several brain areas predicts the high importance of NPY as a neuromodulator. Thus, the effect of NPY on the release of several neurotransmitters such as glutamate, gamma-aminobutyric acid (GABA), norepinephrine (NE), dopamine, somatostatin (SOM), serotonin (5-HT), nitric oxide (NO), growth hormone (GH), and corticotropin releasing factor (CRF) has been examined.²⁷ There is also evidence of its coexistence with NA.²⁸

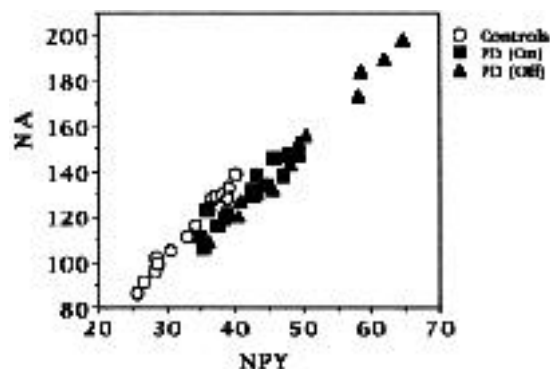


Fig. 3: Correlation between norepinephrine (NA) and NPY in CSF of Parkinson patients (On and Off status) and healthy subjects

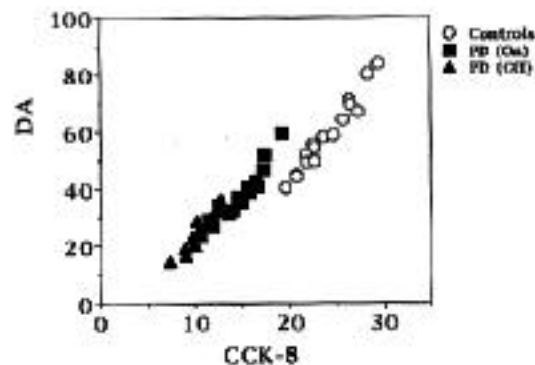


Fig. 4: Correlation between dopamine and CCK-8s in CSF of Parkinson patients (On and Off status) and healthy subjects

Changes in NPY levels have been observed in different pathological conditions such as brain ischemia and neurodegenerative diseases (Huntington's, Alzheimer's disease). Taken together, these studies suggest that NPY and NPY receptors may represent pharmacological targets in different patho-physiological conditions in the CNS. Depression is a multi-factorial process; therefore, it is of interest to study the coexistence of NPY with NA, 5-HT and DA systems relevant to depression since various neurological patients such as those with multiple sclerosis and PD show signs of depression commonly.²⁹

Low levels of NPY in CSF have been reported in depressed patients, particularly in anxiety. However, all our PD patients showed increased CSF level of NPY. The correlation between the levels of NPY shows a linear relationship for PD patients which is similar to healthy controls.

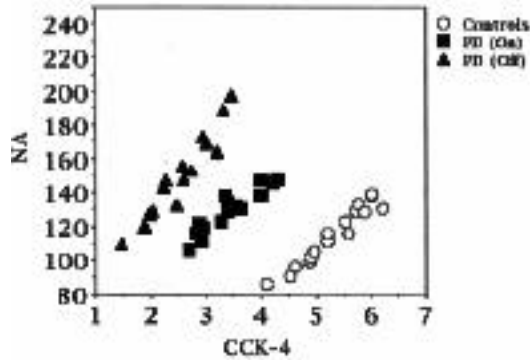


Fig. 5: Correlation between norepinephrine (NA) and CCK-4 in CSF of Parkinson patients (On and Off status) and healthy subjects

Besides NPY, CCK plays a role in physiological, neurological and psychiatric processes. A major role is gut motility, pancreatic secretion and gall bladder contraction. In addition, CCK has effects on locomotor activity,³⁰ and various reward behaviors including cocaine and amphetamine self-administration and food-related reward. Furthermore, CCK plays a role in schizophrenia, anxiety and Parkinson's disease³¹ and these findings have confirmed a neurotransmitter role of CCK. Our knowledge of CCK was greatly enhanced by the recent cloning of cDNAs of rat preprocholecystinin and the CCK receptor.³² CCK-8 is believed to be the most prevalent form of CCK in the CNS. It appears that CCK-8s and various analogs may modulate CNS dopamine neurotransmission and have been studied as a potential antipsychotic agent.³³ Among the other CCK peptides, its tetrapeptide (CCK-4) is angiogenic in humans and considerable amount of data supports its role in anxiety with panicogenic properties with minimal gastrointestinal effects.³⁴ Other functional roles for central CCK (octa peptide, CCK-8s) are as a mediator of satiety responses and as a regulator of sexual and maternal behavior and of seizure activity. The level of CCK-8s showed decreased tendency in PD patients. Figures 4 and 5 show a linear relationship between CSF CCK-8s and DA (Fig. 4) and the similar relationship is seen between CSF CCK-4 and NA in all PD patients (Fig. 5).

Similar to NPY and CCK, Substance P (SP) is a naturally-occurring tachykinin peptide isolated from brain tissues and gastrointestinal tract. In the brain, substantia nigra and basal ganglia contain relatively high amounts of substance P. There is evidence suggesting that substance

P functions as a neurotransmitter. It has been implicated in the patho-physiology of several neuropsychiatric disorders. Substance P may also serve as a useful tool in studying the effects of antidepressant drugs and electroconvulsive therapy.³⁵ However, the contribution of substance P to the understanding of neuropsychiatric disorders is far from clear. SP has received great attention due to its interaction with classical transmitters, especially with 5-HT, NA and DA.³⁶ Very few studies have been conducted to study levels of SP in CSF from the patients with neurological disorders. Figure 6 shows that a linear relationship is found between SP and 5-HT in CSF for healthy subjects as well as in PD patients groups, and irrespective of On or Off status, this relationship and its linearity is intact.

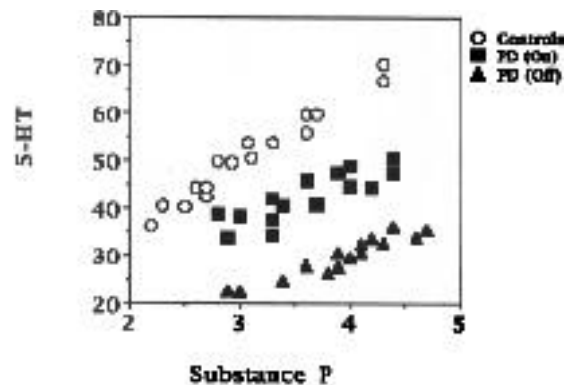


Fig. 6: Correlation between serotonin (5-HT) and Substance P in CSF of Parkinson patients (On and Off status) and healthy subjects.

One possible mechanism may be the difference in the frequency dependence of the various transmitter pools. The release of classical transmitter with low molecular weight is generally believed to depend on the frequency by which action potentials invade the nerve terminal. Brodin et al³⁷ have shown in 1984 that the release of neuropeptide as calculated per pulse recurred at high frequencies compared with what usually is needed to evoke the release of classical small molecule transmitters.

One may conclude that neurodegeneration in PD patients involves some combination of free radical (oxidative) stress, cytostatic Ca²⁺, mitochondrial damage, excitotoxicity, deficiency of vitamins B12, B6 and folate, and role of transition metals especially iron and copper. The eventual result is cell death.

Future therapy in PD is likely to include a 'cocktail' of neuroprotective compounds to interfere with several molecular pathways that lead to neuronal injury. In using therapeutic strategies aimed towards retarding or arresting neuronal death, close attention will need to be paid to quality of life issues. One of the main problems during the

many years of research on PD has been inability to detect the disease early. New methods for early detection and monitoring of disease progression - for example with positron emission tomography in combination with biochemical, pathological or behavioral analysis - will aid in clinical diagnosis as well as future pharmacological treatment that can halt further neurodegeneration and nerve cell death.

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