Excitotoxic mechanisms in non-motor dysfunctions and levodopa-induced dyskinesia in Parkinson's disease: the role of the interaction between dopaminergic and the kynurenine system

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Keywords: Parkinson's disease, kynurenic acid, glutamate, neuroprotection, NMDA receptor, levodopa-induced dyskinesia, excitotoxicity

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Schemes.cdx

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Figure 1. Alterations of the glutamatergic neurotransmission in PD
Fig. 2. The kynurenine pathway
Figure 3. Interactions of KYNA and dopamine

254x190mm (96 x 96 DPI)
Excitotoxic mechanisms in non-motor dysfunctions and levodopa-induced dyskinesia in Parkinson's disease: the role of the interaction between the dopaminergic and the kynurenic system

Abstract: Parkinson's disease is a common progressing neurodegenerative disorder presenting with characteristic motor symptoms. Non-motor dysfunctions and therapy-related complications frequently develop, but are often underdiagnosed and undertreated. Levodopa-induced dyskinesia and impulse control disorders are suggested to share pathophysiological processes and be related to alterations of the glutamatergic neurotransmission. Antiglutamatergic interventions are therefore worth considering: several lines of evidence already indicate their beneficial effect. The kynurenic pathway offers the endogenous glutamate receptor antagonist kynurenic acid, which may provide a promising candidate for future drug development with the aim of assessment of the motor symptoms and therapy-related complications of Parkinson’s disease.

Keywords: Parkinson's disease, kynurenic acid, neuroprotection, NMDA receptor, glutamate, levodopa-induced dyskinesia, excitotoxicity

Introduction

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder presenting with characteristic motor symptoms: rigidity, resting tremor, bradykinesia and postural instability. Epidemiological data indicate that the overall prevalence of PD is around 1.5% with no gender differences; the prevalence increases with the age. The neuropathological background of PD is the progressive selective degeneration of dopaminergic neurons in the substantia nigra. However, as our understanding of the pathophysiological process of PD has improved, it has become evident that, besides the dopaminergic system, the cholinergic, serotonergic, noradrenergic and glutamatergic systems are also affected. Glutamate is the main excitatory neurotransmitter in the human brain, but the excessive stimulation of glutamatergic receptors may lead to neuronal damage, a process known as excitotoxicity.
Excitotoxicity has been implicated in the pathological mechanisms of several
neurodegenerative disorders, including PD\textsuperscript{5,6}.

The pathomechanism of PD has still not yet been fully clarified despite extensive
research, but genetic and environmental factors have both been suggested to contribute.
Most PD cases are sporadic, but a minority of the patients have a clear familial disease.
The role of hereditary factors has been strengthened by epidemiological studies
indicating a significantly higher risk of developing the disease among first-degree
relatives of PD patients\textsuperscript{7-9}. Genetic investigations in families with inherited forms of PD
have identified several gene mutations; these data also promoted the understanding of
the pathogenesis of the disorder\textsuperscript{10,11}. Later, studies suggested that genetic factors may
contribute to a higher risk of PD in the general population as well, and environmental
factors combined with a genetic predisposition may result together in the development
of PD\textsuperscript{12}.

The gold standard of PD therapy currently remains dopamine (DA) replacement with oral
levodopa, which offers good symptomatic relief for the motor symptoms, but can be
associated with therapy-related complications such as levodopa-induced dyskinesia (LID) or
impulse control disorders (ICDs). Non-motor symptoms (NMs) of PD are often unrecognized
and undertreated, although they may have a serious impact on the quality of life. They often
precede the development of motor symptoms and their frequency increases in parallel with the
progression of PD\textsuperscript{13,14}. NMs can be classified into four main groups: autonomic symptoms,
sensory symptoms, sleep disorders and neuropsychiatric symptoms (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Non-motor dysfunctions in Parkinson’s disease</th>
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<tr>
<td><strong>Autonomic symptoms</strong></td>
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<tr>
<td>orthostatic hypotension</td>
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<tr>
<td>gastrointestinal dysfunctions</td>
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<tr>
<td>nausea and vomiting</td>
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<tr>
<td>sexual dysfunctions</td>
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<tr>
<td>bladder dysfunction</td>
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<tr>
<td><strong>Sensory disturbances</strong></td>
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<tr>
<td>anosmia</td>
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<tr>
<td>paraesthesias</td>
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<tr>
<td>pain</td>
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<tr>
<td>taste deficits</td>
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<tr>
<td><strong>Sleep disorders</strong></td>
</tr>
<tr>
<td>rapid eye movement disorder</td>
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<tr>
<td>restless leg syndrome</td>
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<tr>
<td>excessive daytime sleepiness</td>
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<td>vivid dreaming</td>
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<tr>
<td>insomnia</td>
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<td><strong>Neuropsychiatric disorders</strong></td>
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<td>psychosis</td>
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<td>depression</td>
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ICDs include pathological gambling, hypersexuality, compulsive shopping and binge eating. Gambling and hypersexuality show a male predominance, while compulsive shopping and eating are more frequent among female PD patients. The development of ICDs is associated with the use of DA agonists. ICDs are common complications of PD, although they are often underdiagnosed. Epidemiological data indicate the prevalence of ICDs as being between 6 and 18% of PD patients. There are several risk factors which have been associated with the development of ICDs: a younger age, DA agonists or a higher levodopa dosage, a family history of gambling or alcohol problems, impulsivity and depression. The exact pathological mechanisms of ICDs are not yet fully clarified.

The role of interactions of the dopaminergic and glutamatergic systems in the development of therapy-related complications of PD

It has recently been suggested that the pathophysiological background of LID and ICDs is common, and involves alterations of the glutamatergic neurotransmission. Presynaptic changes affecting DA release and postsynaptic changes affecting DA receptors have been described in LID, and are hypothesized to contribute to the development of ICDs too. DA exerts its effect mainly on the medium spiny neurons of the caudate and putamen, which are rich in glutamate receptors. Interactions of DA and glutamate receptors play an important role in the normal function of the basal ganglia, and this fine balance is disrupted in PD. DA replacement therapy results in a chronic intermittent, non-physiological stimulation of DA receptors instead of the physiological tonic stimulation, which leads to changes in the glutamate receptors on the striatal spiny neurons. Experimental data from both animal models and humans indicate that the phosphorylation of glutamate receptors undergoes a change. Phosphorylation of the NMDA receptors is an important mechanism in the regulation of NMDA receptor function. Several kinases have been identified which are able to phosphorylate ionotropic glutamate receptors, including protein kinase A, protein kinase C, Ca(2+)/calmodulin-dependent protein kinase II, Src/Fyn non-receptor tyrosine kinases.
kinases, and cyclin dependent kinase-5. In PD, different signal transduction cascades - the cyclic adenosine monophosphate (cAMP)-protein kinase A-mediated and the calcium-calmodulin-dependent kinase II pathways - are activated, consequently the subunit composition changes and NMDA receptors become phosphorylated. Serin phosphorylation of the NR1 subunit and tyrosine phosphorylation of the NR2 subunit of the NMDA receptors have been described after long-term dopaminergic treatment. The hyperphosphorylation of the NMDA receptors leads to long-term potentiation of their sensitivity. Another important kinase involved in the phosphorylation of NMDA receptors and the mediation of dopamine-dependent signaling processes is glycogen synthase kinase 3-beta (GSK3β). Activation of GSK3β has been proved to inhibit presynaptic glutamate release, alter the expression of NMDA receptors and under hyperdopaminergic conditions, affect synaptic NMDA-mediated currents. GSK3β has been described to be involved in several pathogenic processes of PD, including oxidative stress, protein aggregation and neuroinflammation.

These alterations result in hypersensitivity towards DA and the development of motor fluctuations. The possible role of the connection of DA and glutamate receptors in the pathogenesis of ICDs has been strengthened by a genetic study which found an association of the GRIN2B gene with ICDs. GRIN2B is responsible for the synthesis of the NR2B subunit of NMDA receptors, which is the predominant form of the striatal NMDA receptors.
The kynurenine pathway and its interaction with the dopaminergic system

The kynurenine pathway (KP) is the main route of the tryptophan (Trp) metabolism, responsible for more than 95% of Trp degradation (Fig. 2.)\textsuperscript{36, 37}. It has been recognized already in the 1980s that the enzymatic cascade of the KP generates several neuroactive compounds\textsuperscript{38, 39}. The first, rate-limiting step of the KP is the enzymatic degradation of Trp, this step catalyzed by indoleamine-2,3-dioxygenase (IDO). The KP divides into two main branches at a central intermediate, L-kynurenine (KYN). The first arm of the pathway is the conversion of KYN into kynurenic acid (KYNA). KYNA synthesis can be attributed to the action of kynurenine-aminotransferases (KATs), so far 4 subtypes of this enzyme are known, which have slightly different biochemical profile\textsuperscript{40}.

The other branch of the KP begins with the conversion of KYN by kynurenine-3-monooxygenase (KMO) into 3-hydroxy-kynurenine. This is further metabolized by multiple enzymatic steps, gives rise to the neurotoxic quinolinic acid (QUIN) and finally ends at the production of the essential coenzyme nicotinamide adenine dinucleotide (NAD).

![Fig. 2. The kynurenine pathway](image-url)
KYNA is a broad-spectrum endogenous inhibitor of ionotropic excitatory amino acid receptors. It has the highest affinity for the N-methyl-D-aspartate (NMDA) type glutamate receptors, exerting its effect mainly by binding to the strychnine-insensitive glycine-binding site, but at a higher concentration it can also bind to the glutamate-binding site. On another type of glutamate receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, KYNA exerts a concentration-dependent dual effect: in a higher, micromolar concentration it is an inhibitor, while in a nanomolar concentration it acts as a facilitator. KYNA has also been described to be a non-competitive inhibitor of the presynaptic α7 nicotinic acetylcholine receptors thereby reducing presynaptic glutamate release. Later investigations could not confirm this inhibitory potential. However, KAT-deletion and consequently a loss of KYNA resulted in an increased sensitivity of α7 nicotinic acetylcholine receptors, suggesting a regulatory role of KYNA on them. Currently, the possible role of KYNA on α7 nicotinic acetylcholine receptors is not clearly understood and further research is needed to describe any potential effect.

It was subsequently confirmed that KYNA is an agonist of the previously orphan G-protein coupled receptor GPR35, which has also been suggested to contribute to the reduction of extracellular glutamate in the brain. The complex modes of actions suggest that KYNA has an important neuromodulatory role in the central nervous system and it is involved in the regulation of glutamatergic and cholinergic neurotransmission (Table 2).

<table>
<thead>
<tr>
<th>Table 2. Receptor targets of KYNA and its effects on them</th>
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<tr>
<td>NMMA receptors</td>
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<tr>
<td>AMPA receptors</td>
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<tr>
<td>α7-nicotinic acetylcholine receptors</td>
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<td>GPR35 receptor</td>
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Increasing evidence confirms the neuroprotective capacity of KYNA in different conditions, such as QUIN, NMDA and glutamate-induced toxicity or ischaemia. On the other hand, QUIN is a neurotoxic compound which exerts its deteriorating effect mainly via NMDA agonism, but also contributes to the generation of free radicals, promotes lipid peroxidation and decreases antioxidant capacity. Interestingly, in contrast with KYNA, QUIN enhances glutamate release.
An important aspect regarding the effects of KYNA is its close connection with the dopaminergic system. Elevations of KYNA levels result in the inhibition of dopamine release. This effect is suggested to be mediated predominantly by the α7-nicotinic acetylcholine receptors, but at higher concentrations this effect may be due to the inhibition of glutamate receptors \cite{62,63}. Conversely, levodopa substitution has also been confirmed to reduce KYNA levels \cite{64}. (Fig. 3.)

These results further strengthen the concept that KYNA has an important role in the regulation of glutamatergic, cholinergic and dopaminergic neurotransmission, which may also have important implications for the pathophysiology of PD. Alterations of KYNA are able to modulate DA levels, while reduced KYNA levels may also increase vulnerability towards excitotoxic effects by enhancing glutamatergic influences. \cite{65} Alterations in the delicate balance of neuroactive kynurenines have been confirmed in several neurodegenerative diseases, including Alzheimer’s disease, multiple sclerosis and Huntington’s disease \cite{66-69}. KP changes have also been described in PD. Kynurenine/Trp ratios were increased, while serum Trp levels were decreased in PD patients as compared with healthy controls \cite{70}. Ogawa et al. have measured reduced concentrations of KYN and KYNA in several brain regions of PD patients, including the frontal cortex, putamen and substantia nigra \cite{71}. They also confirmed a significantly increased 3-OH-KYN levels in the putamen and substantia nigra of PD brains; this compound may contribute to the oxidative stress and consequently to the neuronal damage \cite{71}. KP alterations have also been measured at the periphery: in the plasma of PD patients, the KYNA level and the KAT activity have been found to be decreased \cite{72}.
**Therapeutic opportunities by glutamate antagonism**

Memantine, a weak NMDA antagonist, has been revealed to have beneficial symptomatic effects for dementia associated with PD and, interestingly, it may additionally improve motor symptoms. Case reports suggested that memantine may also improve LID. So far there is no data concerning its therapeutic effect on non-motor symptoms or ICDs.

Another anti-glutamatergic agent, amantadine has been confirmed to improve LID symptoms both as acute and as chronic therapy. It was proved that the beneficial effect of amantadine is long-lasting, and is sustained even after 1 year of continuous therapy. The beneficial effect of amantadine on LID is suggested to be modulated at least in part by NMDA inhibition. Amantadine has been already tested for the therapeutic management of ICDs with promising results. A small study has revealed that amantadine is capable of reducing pathological gambling in PD patients. The effect of amantadine was recently confirmed by another small-scale study, which described the behavioral background of this effect. The possible therapeutic effect of amantadine was strengthened by other studies involving patients with pathological gambling without PD. Case reports have described the beneficial effect of amantadine on punding as well, which is another complication of dopamine-replacement therapy.

These results suggest that anti-glutamatergic therapies may be valuable options for the management of not only the motor symptoms, but also of the therapy-related complications of PD. KYNA is the only known endogenous NMDA antagonist, which has been suggested to provide symptomatic treatment and also neuroprotection. In an experimental PD model, KYNA was able to alleviate parkinsonian motor symptoms. There is growing evidence indicating that the hypersensitivity of NMDA receptors plays an important role in the development of LID and other complications of PD, and the NMDA antagonist KYNA may therefore be beneficial for the treatment of these complications too. Elevation of the brain KYNA levels has been already examined in experimental PD models. KYNA itself can cross the blood-brain barrier poorly, but the peripheral administration of its precursor, KYN, together with the organic acid transport inhibitor probenecid, results in significant brain KYNA content elevations. This treatment proved to be neuroprotective in a 6-hydroxydopamine model of PD. Another possibility is the synthesis of kynurenine derivatives, in another animal model of PD, the neuroprotective effects of several synthetic kynurenines were confirmed. Another option to achieve a KYNA elevation would be to influence the enzymatic machinery of the KP; accordingly, KMO inhibition results in a shift
towards the production of KYNA. This method resulted in the improvement of LID, while the antiparkinsonian effect of levodopa was not diminished\textsuperscript{91,92}.

There are not yet data regarding the effects of KYNA on the non-motor complications of PD, but the promising results with amantadine, and the fact that KYNA is able to improve LID, suggest that KYNA may be of therapeutic value for these conditions too. Further investigations are worth considering to assess the potential therapeutic benefits of KYNA in PD. From this aspect it is of interest to produce synthethic KYNA derivatives which may have more favourable pharmacokinetic properties than those of KYNA, such as an improved receptor selectivity or an improved blood-brain barrier penetration. Several synthethic KYNA analogues have already been developed to achieve neuroprotection\textsuperscript{93}.

**Syntheses of kynurenic acid derivatives**

The general procedure for the synthesis of 4-hydroxyquinolinic acid can be achieved by a modified Conrad-Limpach method starting from the commercially available substituted aryl amines \textsuperscript{1} \textsuperscript{93,94}. The first step involves enamine bond formation by using dimethyl acetylenedicarboxylate or diethyl acetylenedicarboxylate resulting in 2 (Scheme 1).

![Scheme 1](https://mc04.manuscriptcentral.com/crmc)

The intermediate fumarate 2 is then cyclized at high temperature, yielding 4-hydroxy-2-carboxylates 3 respectively. For 3 the enol-oxo tautomerism is possible. This equilibrium is shifted to the oxo form when C-2 contains an ester function, while the presence of a carboxylic group at C-2 indicate the enol form (Scheme 1). For the transformations of the 4-hydroxy-quinoline derivatives, the free acids (KYNA derivatives) are needed. The hydrolysis of the esters is generally performed in methanolic alkali media, followed by acidification with HCl, resulting in kynurenic acid derivatives 4 (Scheme 1).

The transformations of KYNA derivatives can be achieved in different ways: by the transformation of the synthetically active hydroxy group at position 4 or by the conversion of the carboxylic function at position 2\textsuperscript{93}. 

\textsuperscript{9}
The aim in the last few years has been to introduce a cationic centre in the side-chain so as to have good water solubility and better penetration ability of the products through the blood-brain barrier. The coupling between KYNA and 2-dimethylaminoethylamine was first achieved by using N,N’-diisopropylcarbodiimide in the presence of 1-hydroxybenzotriazole hydrate, yielding 5 (Scheme 2).

Scheme 2.

The excellent biological activity of 5 led us to prepare a number of analogues. Several of them is shown in Scheme 2. For example, by using 2-diethylaminoethylamine as starting amine, 6 was synthesized as a diethyl analogue of 2 (Scheme 2), and analogues 7, 8 and 9, containing the tertiary nitrogen in different ring systems, were prepared by reacting KYNA with 2-pyrrolidinoethylamine, 2-piperidinoethylamine and 2-morpholinoethylamine, respectively.

The synthethic KYNA analogues have already been tested in several animal models of neurological diseases with promising results. In an animal model of transient global forebrain ischaemia, KYNA exerted a neuroprotective effect by reducing the hippocampal CA1 pyramidal cell loss. The analogue was effective both as a pre-treatment and as post-treatment. In a transgenic mice model of Huntington’s disease, the same analogue proved not only to ameliorate the motor symptoms but it also significantly, by more than 30%, prolonged the survival of the animals. The neuroprotective effect was also confirmed histologically: the KYNA analogue was able to
prevent neuronal atrophy in the striatum of the transgenic animals. As KYNA is an NMDA antagonist, an important concern would be the possible interference with the physiological glutamatergic functions. However, behavioral studies with this novel KYNA analogue confirmed, that in the same dose in which it proved to be neuroprotective, it did not induce any cognitive side effects.

Conclusion
The therapeutic management of PD through dopamine replacement is associated with therapy-related complications, which may have a more severe impact on the quality of life of patients than the parkinsonian motor symptoms. Alterations of the glutamatergic neurotransmission are implicated in the development of both LID and ICDs, and antiglutamatergic interventions may therefore be beneficial for these complications. KYNA as an NMDA antagonist compound, which is also able to influence dopaminergic neurotransmission may be a promising candidate for future drug development.

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Conflict of Interest
The authors have no conflict of interest to report.
References


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