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Review

Morbus Parkinson – A synaptic disorder?

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ABSTRACT

Currently, the pathophysiology of Parkinson's disease is explained by a loss of mainly dopaminergic nerve cells which causes a neurotransmitter deficiency. In the final stage, the substantia nigra shows a marked loss of neurons in Parkinson's disease. In some of the remaining neurons, Lewy bodies can be found and serve as pathological hallmark of disease. These Lewy bodies are composed mainly of aggregated α-synuclein, a protein, physiologically found at pre-synapses. Lewy bodies were thought to be the pathophysiologically relevant form of α-synuclein pathology because their presence coincides with neuron loss in the substantia nigra.

As the clinical symptoms suggest a synaptic pathology, involvement of the pre-synapses in the α-synuclein aggregate pathology has been found recently. One to two orders of magnitude more α-synuclein aggregates than Lewy bodies or Lewy neurites can be found at pre-synapses. A degeneration of dendritic spines associated with the synaptic α-synuclein aggregate pathology has been shown to occur in human disease. In experiments using transgenic mice or cell cultures, mild (two- to threefold) overexpression of α-synuclein caused an altered vesicle turnover and led to a reduction in neurotransmitter release. Different approaches linked these alterations to pre-synaptic aggregation of α-synuclein.

These findings may change the pathophysiological concept of Parkinson's disease fundamentally. Not nerve cell loss but the synaptic dysfunction of still existing nerve cells should become the focus of attention. Future strategies for therapies should concentrate on the maintenance of synapses rather than focusing on the mechanism of cell death or cell replacement strategies.

Contents

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Lewy bodies in the pathophysiology of Parkinson's disease

The current pathophysiological hypothesis for the disease mechanism of Parkinson's disease is that loss of dopaminergic neurons result in a depletion of the neurotransmitter dopamine in the striatum, which in turn causes the motor symptoms bradykinesia, tremor, rigidity and postural instability [1]. In Parkinson's disease, Lewy bodies are mainly found at predilection sites of neuronal loss, i.e. the substantia nigra and locus coeruleus. These findings are the hallmark pathology seen in the final stages of the disease. The coincidence of both neuron loss and Lewy bodies led to the conclusion that Lewy bodies were the pathophysiologically relevant form of α-synuclein pathology responsible for the disease [2].

The incidence of Lewy bodies in brains of asymptomatic individuals increases with advanced age. This raises the question of whether Lewy bodies reflect presymptomatic Parkinson's disease, as proposed by Dickson et al. [3], or are a feature of normal aging [4]. Gibb reported an age-dependent increase in the prevalence of Lewy bodies from 3.8% to 12.8% between the sixth and ninth decade of age. This is an amount that exceeds the prevalence of Parkinson's disease by about three- to sixfold [5]. Many other studies show similar findings (for review see [6]).

Because the number of Lewy bodies in patients with mild to moderate loss of neurons in the substantia nigra is higher than in patients with severe neuronal depletion, Lewy body-containing neurons have been assumed to be the dying neurons [7]. In contrast, it has been shown recently that neuronal dysfunction and nerve cell loss of nigra-neurons may precede the Lewy pathology [8]. The presence of Lewy bodies does not predispose substantia nigra neurons to undergo apoptotic cell death to a greater degree than the general population of substantia nigra neurons, and most neurons that undergo cell death do not contain Lewy bodies [9]. Substantia nigra neurons, whether they contain Lewy bodies or not, are similarly affected, for example by morphological dendritic abnormalities or biochemical changes, indicating that the neurons in general are involved in the disease process [10–13].

Consequently, attempts to correlate the density of either cortical or brain stem Lewy bodies with clinical disease symptoms in Parkinson's disease and DLB were not successful. Most studies failed to correlate Lewy body density with early onset of disease, disease duration, symptoms at onset, visual hallucinations, delusions, recurrent falls, severity of parkinsonism, presence or absence of cognitive fluctuations or cognitive decline [14–18]. The presence of symptoms may be related to the involvement of defined regions as measured by the occurrence of Lewy bodies [19–21]. In a percentage of Parkinson's patients who developed dementia, however, no Lewy bodies could be detected in cortical areas or in other areas outside the brain stem [22,23]. These findings indicate that the pathophysiology of the neurodegenerative process can hardly be explained by Lewy bodies. It is most likely that the Lewy body formation is a process for detoxification of α-synuclein aggregates located at a harmful site in the neuron [24]. Localization, composition and ultrastructure indicate Lewy bodies being formed in an aggresome-related process. This supports the notion that Lewy bodies are a compartmentalization of protein aggregates to protect the cell [25].

Physiologically, α-synuclein is a protein localized in presynapses, that promotes the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-complex assembly in the form of a chaperone activity [26], and that maintains the size of the presynaptic vesicular pool as well as the vesicle recycling [27–30]. Its function is important for neurotransmitter release [31,32], especially for dopamine release [33–37].

Clinical findings suggest synaptic pathology in Parkinson's disease

The clinical symptoms of Parkinson's disease suggest that a failure of synapses is the pathophysiological mechanism of disease. Tremor at rest, rigidity, akinesia/bradykinesia and postural instability are the four cardinal features in Parkinson's disease [38]. Akinesia/bradykinesia is assumed to be the result of a disruption of motor cortex activity (for review see [38]), while tremor and rigidity were explained with nigrostriatal dopaminergic deficits. Dopamine replacement treatment was the major breakthrough for Parkinson's disease patients in the last century [39]. Various studies of in-vivo imaging of synaptic functions of the CNS found pre-synaptic neurotransmitter deficiencies in Parkinson's disease (overview in [40]). All of these findings indicate that in Parkinson's disease the degenerative process is located at the pre-synapse [41] and results in a neurotransmitter deficiency syndrome. When α-synuclein aggregation is causally linked to the pathophysiology of disease, it can be assumed that the aggregation takes place at the synapse.

Are α-synuclein aggregates related to synapses?

To test this hypothesis we used paraffin-embedded tissue (PET) blotting, the most sensitive known method for the topographical detection of protein aggregates [42]. Paraffin-embedded tissues were cut the same way as for conventional histology, but the slides were placed onto a nitrocellulose membrane. By protease digestion involving the use of detergents, protein aggregates were mobilized from the tissue, bound to the nitrocellulose membrane, and antigenic epitopes in the aggregates were demasked [43]. Protease-resistant aggregates were detected by antibodies and visualized by a formazan reaction [42]. For technical reasons, we investigated the synaptic α-synuclein pathology first in cortex samples of DLB patients. As assumed in our hypothesis, we were able to detect a significant amount throughout the cortex of α-synuclein aggregates that appear to be much smaller than Lewy bodies. The micro-aggregates were most dense in the cingulate cortex and their distribution was identical with that of the synaptic protein synaptophysin, suggesting a synaptic localization. The same can be observed at predilection sites in Parkinson's disease [44].

We were able to quantify biochemically that the amount of synaptic α-synuclein aggregates exceeds that of Lewy bodies or Lewy neurites by one to two orders of magnitude. The problem for biochemical analyses of α-synuclein aggregates is that these aggregates are highly insoluble [45]. Even after extraction with guanidinium hydrochloride, urea or formic acid, the majority of aggregates get stuck at the bottom of the loading pocket of the gel for electrophoretic separation in Western blot analysis. Thus a quantification of α-synuclein aggregate content is impossible with Western blot [46].

We solved the problem by using a protein aggregate filtration (PAF) assay. Based on previously described methods [47,48], the sucrose gradient fractions were sucked through a 200 nm pore-size membrane, and the aggregates were quantitatively retained and separated from soluble α-synuclein [46]. Undesirable binding of soluble proteins to the membrane was blocked with an amphiphilic polymer [49]. With this method it was possible to detect α-synuclein aggregates reliably and most sensitively and to quantify the amount of aggregates, as shown in dilution series [46].

Where are the α-synuclein aggregates localized at the synapse?

The question of where the α-synuclein micro-aggregates were localized at the synapse was addressed by analyzing subcellular