Review

Does circadian autophagy have a protective or degenerative role in neurodegenerative disease?

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Abstract

Introduction
Dementia is a non-curable, terminal neurodegenerative disease that affects 50 million people globally, posing a burden to patients and their families.[1] Autophagy, the process of clearing cellular debris, is a possible contributor to dementia.[2] This literature review explores the homeostatic role of autophagy in sleep and its implications in neurodegeneration.

Methods
The primary research was conducted through the NCBI platform (PubMed Central) to obtain the latest original research papers and reviews from the last 10 years. Records were identified based on whether they contained the phrases "dementia", "autophagy" and "sleep". Fifteen records were identified and short-listed based on their title and abstract. Studies were only taken forward if they focused on the neuronal mechanisms, genetic, and hormonal pathways of dementia and autophagy. Eleven studies were included in the final review.

Results
Studies demonstrate that autophagy has a neurodegenerative role in the setting of chronic sleep fragmentation (CSF) and vascular occlusion. The upregulation of certain molecular pathways leads to the accelerated formation of autophagolysosome vesicles and the accumulation of abnormal protein aggregates. In Parkinson's Disease (PD), autophagy is protective because it is contained within the microglia of neurons. A possible link exists between autophagy and circadian rhythms, which is influenced by modafinil and melatonin.

Conclusion
There is conflicting evidence on the role of autophagy as a possible contributor to neurodegenerative disease. Further research is needed to definitively conclude the relationship between autophagy, sleep and neurogenerative disease, and whether these autophagy regulatory pathways can be targeted as treatment modalities for the same.

Abbreviations

Aβ – Amyloid βeta
AD - Alzheimer's disease
APP - Amyloid precursor protein
ATG - Autophagy Related Genes
CSF – Chronic Sleep Fragmentation
ER – Endoplasmic Reticulum
LPS – lipopolysaccharide
MPTP - 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PD – Parkinson's Disease

Introduction

The main features of dementia are impairment of memory and cognition, which are associated with ageing. The pathogenesis of the disease begins years before it clinically manifests. This is attributed to the accumulation of cellular debris overtime, ultimately leading to neuronal injury and degeneration.[3] In healthy individuals, this cellular debris is cleared by a catabolic process known as autophagy. It involves the lysosomal degradation of misfolded proteins and damaged organelles through the formation of an

autophagolysosome.[2] Under physiological conditions, a basal level of autophagy occurs to maintain the homeostasis of neurons by clearing cellular debris. This prevails during sleep, under the influence of melatonin.[4] Under pathological conditions, the accumulation of abnormal protein aggregates and neurotoxins induces excess autophagy, which can lead to endoplasmic reticulum (ER) stress and mitochondrial damage. Multiple proteins and hormones, including melatonin, play a protective role against neurodegeneration by regulating this process.[4] A disruption in autophagy pathways has been implicated in many neurodegenerative diseases. This review explores the pathways of autophagy that are induced during sleep, and both their neuroprotective and neurodegenerative role in the pathogenesis of neurodegenerative disease.

Method

A primary search of full-text articles was conducted on PubMed to determine the final chosen publications for the review, as detailed in the adjunct below (**Figure 1**).

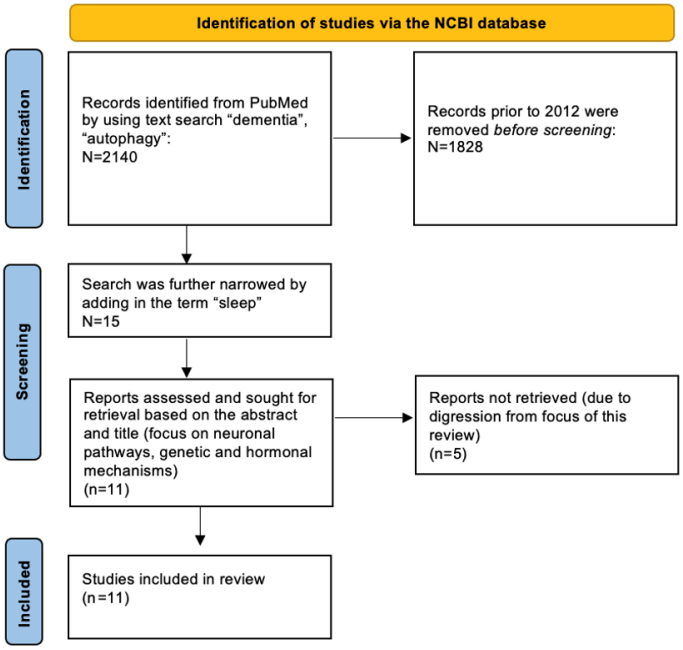

Figure 1. Flow diagram for the selection of studies for this review.

Discussion and results

Autophagy in chronic sleep fragmentation (CSF)

CSF impairs memory function due to a pathogenic process like that seen in Alzheimer's disease (AD). AD is the most common type of neurodegenerative disease, and is characterised by memory loss, spatial learning disability and behavioural changes in advanced disease.[5] A study applied a CSF model in wild-type mice with no genetic predisposition to dementia. After two months, investigations revealed significant cognitive impairment in mice with β-amyloid accumulation in the cortex and hippocampus.[5] This can be attributed to an imbalance between the formation and clearance of β-amyloid protein (autophagolysosome pathway dysfunction). In normal conditions, β-amyloid is cleared by internalisation of Amyloid precursor protein (APP) into the cell via early and late endosomes (detected by the protein markers Rab5 and Rab7, respectively). It is degraded into β-amyloid by APP secretase, which fuses with lysosomes for degradation. CSF dysregulates this pathway and leads to the accumulation of abnormal vesicles within the cell. Western blot and immunofluorescent staining, lab techniques used to detect proteins in tissue samples, indicated enhanced expression of Rab5 and Rab7 in the brain of wild-type mice after CSF. Additionally, Lamp 1 and LC3B (lysosome and autophagosome markers respectively), and Beclin 1 and UVRAG (autophagy positive regulators) were also detected (**Figure 2**). The presence of autophagy proteins in the setting of CSF indicates accelerated formation of autophagolysosome vesicles. As a result, an imbalance between the formation and degradation of β-amyloid occurs, ultimately leading to intracellular β-amyloid being released into the extracellular space to form A β plaques.[5]

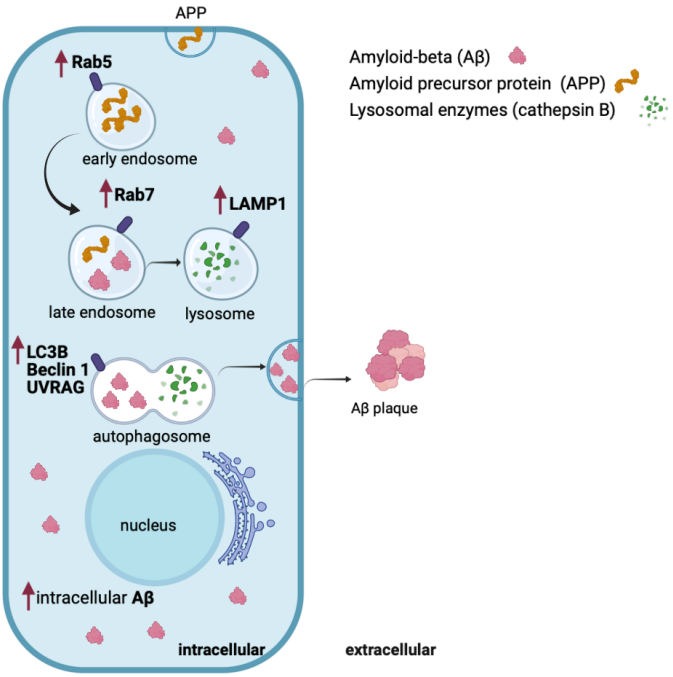

Figure 2. Accumulation of AB extracellularly due to the acceleration of autophagosome formation.

Autophagy and vascular occlusion

This theory is supported by another study exploring the role of Beclin 1 and cathepsin B (lysosomal enzyme) in vascular dementia (VaD). VaD is characterised by problems with reasoning, planning and judgement due to impaired blood flow to the brain.[6] The Pulsinelli four-vessel occlusion method was used to mimic vascular dementia in rats, by occluding the vertebral arteries whilst controlling the collateral circulation to the brain.[7] This induced ischemia in different cerebral regions, with the hippocampus being the most sensitive. The Morris water maze, a navigation test, was used to assess performance in learning and memory. The results indicated that vascular dementia induces expression of Beclin 1 and cathepsin B, increasing autophagy and decreasing performance on the Morris water maze. In a subset of subjects, wortmannin (an autophagy inhibitor) was administered. Less neuronal injury and higher performance on the Morris water maze was reported in this group.[8] Although vascular dementia due to hypoxic damage to neuronal tissue is different to the proteinopathic damage seen in other forms of neurodegenerative disease, the implication of autophagy is this process indicates the complexity of the process.

The effect of modafinil on autophagy

A similar effect is seen in with the administration of modafinil, a wakefulness promoting agent. A study found accumulated Nissl bodies, the equivalent of endoplasmic reticulum in neurons, and aggravated apoptosis in hippocampal neurons of sleep deprived mice. These changes were due to a decrease in the Bcl2/Bax ratio, which leads to the release of apoptogenic molecules from the mitochondria.[9] On administration of modafinil, excessive autophagy was attenuated in vitro and in vivo, restoring spatial memory in these mice. Modafinil phosphorylates the PI3K/Akt mTOR/P70S6K signal pathway (**Figure 3**), and suppresses the expression of L3B, Beclin-1, and P62. This alleviates the neuronal apoptosis that occurs when the formation of autophagolysosome vesicles exceed the lysosome's degenerative capacity.[10]

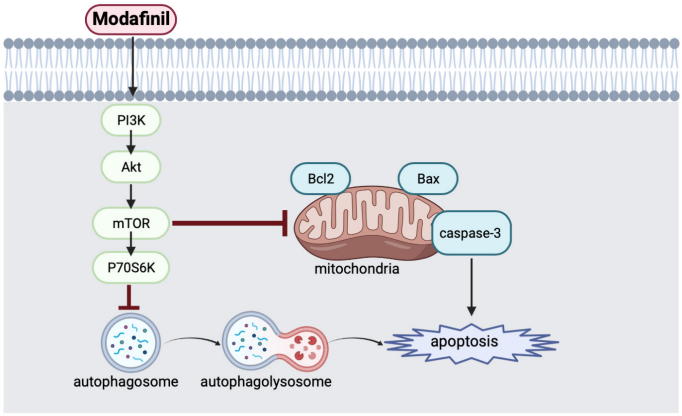

Figure 3. The effect of modafinil on the PI3K/Akt mTOR/P70S6K signal pathway.

Autophagy and Parkinson's disease (PD)

PD is a progressive neurodegenerative disorder which leads to bradykinesia, rigidity, and tremor.[11] Its pathogenesis is unclear but mainly involves a compromise in mitophagy, the process of removing defective mitochondria, and the aggregation of α-synuclein protein leading to loss of dopaminergic neurons.[12] In opposition, autophagy has been shown to have neuroprotective effects in PD and other forms of neurodegenerative disorders involving the accumulation of α-synuclein such as Lewy body dementia.

The clearing of α-synuclein aggregates in mice injected with the human form of this protein is done by microglia and is mediated by the TLR4-NK-κB-p62 pathway (**Figure 4**). Immunostaining indicated an intracellular increase in p62 levels, an autophagy receptor, but unaltered LC3 intracellular levels. The LC3 was localised within microglia containing α-synuclein. This suggests that autophagy was restricted and selective to α-synuclein rather than globally upregulated within the brain. The binding of the p62 receptor to ubiquitin-linked α-synuclein protein likely mediates the process.[13] In doing so, neurotoxicity by α-synuclein is prevented.

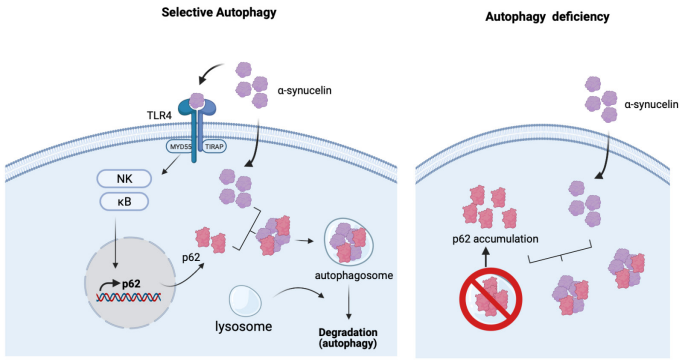

Figure 4: α-synuclein selective autophagy vs autophagy deficiency.

The effect of melatonin and circadian rhythms on autophagy

The role of p62 and LC3 is further explored in a study looking at excessive autophagy in the hippocampus of sleep-deprived mice. Sleep deprivation modulated the expression of glutamate receptors and affected hippocampal synaptic plasticity, reducing the performance of sleep-deprived mice in a Y maze test. On Western blot analysis, the expression of p62 was downregulated in the hippocampus of sleep-deprived mice. Meanwhile, the number of autophagic vacuoles observed under transmission electron microscopy increased, and the expression of Beclin-1 and LC3 proteins was upregulated.

Melatonin, a sleep-promoting hormone produced in response to darkness, is an important regulator of our circadian rhythms (24-hour internal clocks).[15] Lack of melatonin could be a possible contributor to neuroinflammation, as seen in mice who underwent lipopolysaccharide (LPS) treatment. LPS treatment causes neuroinflammation, inducing depressive like behaviours and cognitive impairment. This is due to increased levels of cytokines and NF-κB phosphorylation, causing dysregulation of Autophagy Related Genes (ATG).[16] These effects are modulated through the expression of the FOXO3a transcription factor. FOXO3a promotes autophagy by upregulating FOXO1, increasing activity in the PI3K-AKT1 pathway responsible for neuronal apoptosis.[17] When melatonin was administered to the LPS treated rats, cognitive impairment and depression were improved. These anti-depressive effects and cognitive enhancement were modulated through an increase in FOXO3a signalling pathway, upregulating the expression of ATG genes. The exact mechanisms of how melatonin interacts with the FOXO3a transcription factor remains unclear.[16]

Additionally, melatonin can also exert its neuroprotective effect through autophagy inhibition. An experimental animal model of PD was induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP damages dopaminergic neurons by upregulating autophagy through CDK5. These changes were salvaged by the administration of melatonin which led to the knockdown of CDK5. This reduced autophagy-related dopaminergic neuron loss and dyskinesia symptoms in the PD animal model.[18]

Further evidence suggests that autophagy is regulated in a rhythmic pattern, similar to the 24-hour internal clock in humans. This is termed "circadian autophagy". A number of circadian transcriptional regulators upregulate the expression of autophagy genes. One of which is the C/EBPβ transcription factor. In cultured cells, C/EBPβ directly binds to the promoter regions of the ATG genes and activates their transcription.[19] This indicates a possible relationship between circadian rhythms and autophagy.

Limitations

The papers reviewed provide strong evidence for multiple molecular pathways in autophagy and neurodegenerative disease, but some limitations involving the methodology exist. Despite the use of navigation models and maze tests such as the Morris water maze and the Y maze test, it is difficult to quantify cognitive function and depressive symptoms in the study subjects. Furthermore, the significance of using mice in some studies and rats in others is unclear, and it is uncertain whether underlying differences in the neuroanatomy of each species influenced the results.

Environmental triggers and psychological factors such as educational background and underlying mental illness also heavily influence the development of neurodegenerative disease in humans. Despite attempting to control baseline cognition at a neuronal level in the subjects studied via the use of agents such as LPS and MPTP, it is nearly impossible to account for these individual differences in humans. This impacts the ability to translate findings directly to the human population.

Conclusion

The link between autophagy and neurodegenerative disease has demonstrated that autophagy acts as a protective mechanism against neurodegeneration, whilst creating a damage pathway that can lead to programmed cell death. The transcriptional regulation of this catabolic process in a diurnal manner is heavily influenced by circadian rhythms and melatonin. Whether sleep is protective against neurodegeneration is controversial. This is due to the conflicting evidence on the protective roles of stimulants and wakefulness-promoting agents against neuronal apoptosis. Further research is needed to definitively conclude the subtle interplay between autophagy, sleep and neurodegeneration, and whether

these autophagy regulatory pathways can be targeted to treat neurodegenerative disease.

Acknowledgements

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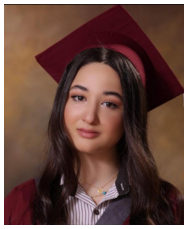

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I am currently a 4th year medical student at Cardiff University. The cellular pathways involved in neurodegeneration are a fast-growing field in neurology. Understanding how these pathways go array in disease can enable us to translate that knowledge into real-life solutions that help everyday people suffering from neurodegenerative diseases. This is why I chose this topic as part of my year 2 SSC project at Cardiff. I hope to contribute more to this field throughout my medical career and look forward to the exciting discoveries yet to be made in the future!