Parkinson's Disease: Molecular Mechanisms and Treatments

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A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2012

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial
fulfillment of the requirements for graduation from the
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Abstract

Parkinson's disease is a motor system disorder that is caused primarily by the loss of dopamine-producing brain cells. The most affected brain structure is the pars compacta of the substantia nigra. This area of the brain is essential to the control of voluntary movement, and so its impairment leads to symptoms such as tremors, rigidity, and impaired balance. The neuronal protein alpha-synuclein has been shown to be heavily involved in the pathogenesis of the disease at the cellular level. The currently available treatments for PD mainly target dopamine regulation, and there been no cure developed for the disease at present. New treatments must be explored by an evaluation and synthesis of the current research and should be adjusted for each patient individually.

Parkinson's Disease: Molecular Mechanisms and Treatments

The biological mechanisms and treatment pathways for Parkinson's disease (PD) are slowly being uncovered. The molecular basis of the disease and its various causes are the fundamental starting point of this process. In order to understand how the symptoms of Parkinson's present and how they can be targeted, it is important to determine how the molecular pathology affects the PD patient on a macroscopic level. The currently available treatments for PD are continually being improved and expanded upon since there has been no cure developed for the disease at present. Possible new avenues for treatment must be explored by an evaluation and synthesis of the current research and therapy options available.

**Introduction and Justification of Research Approach**

Parkinson's disease is a disorder of the motor system. It is caused primarily by the loss of dopamine-producing brain cells.  There are four main symptoms of PD: tremors, rigidity, bradykinesia, and impaired balance and coordination. All of these symptoms have a molecular- and cellular-level basis, much of which has yet to be completely revealed. The most notable brain structure damaged in Parkinson's disease is the pars compacta, a part of the substantia nigra. The neurons in this area of the brain are part of a pathway for the control of voluntary movement and they use the neurotransmitter dopamine. When dopamine is not present at normal levels, as in the case of PD, this causes an individual's ability to coordinate movement to be significantly impeded.

Because of the diversity and variability inherent in the clinical manifestation of Parkinson's disease, the diagnoses are often broadened into a spectrum of disorders known as *parkinsonisms.* Furthermore, the diagnostic criteria of the disease have changed

over time. These criteria have been developed based on both the symptoms that are

observed in Parkinson's disease patients and their underlying molecular causes.

Confirmation of the presence of the disease is sometimes not apparent until the

patient has died and a postmortem examination has been made. Although strict criteria

have been developed and accepted for the diagnosis of Parkinson's disease, there still

exist certain *gray areas* in diagnostics. Certain specifics of the disease currently qualify

as *parkinsonisms*, but these aspects deserve attention due to their relevancy and

correlation to the purported *Parkinson's disease*.

Parkinson's disease has a variety of genetic sources. However, several epigenetic

causal factors have been identified as well. There is evidence that one of these factors is

an increase in the formation of reactive oxygen species. These reactive species can

originate both inside and outside the mitochondria (Zhou, Huang, & Przedborski, 2008).

Another causal factor may be toxins in the environment. For example, a

contaminant found in some illegal drugs in the 1980s, methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP), was found to lead to the development of parkinsonism in

drug users. MPTP is soluble in lipids and so it is able to cross the blood-brain barrier. The

toxin is then modified inside the brain and is recognized by the dopamine transporter.

This is extremely detrimental to the neurons because the molecule binds to NADH

dehydrogenase and essentially terminates the electron transport chain. Starved for ATP,

these cells shortly die.

Besides the environmental causes of Parkinson's and parkinsonisms, there are

several common genetic mutations that can occur that lead to the disease. Possible sites

for a mutation include PARK1, the gene that codes for α-synuclein, and PARK2 through

PARK12. There is strong evidence that α-synuclein plays a principal role in the development of Parkinson's disease. However, due to the delicate complexity of PD, there are likely to be many different important proteins involved in the process and these principal proteins may differ in the extent of their role from patient to patient. Each of the gene products transcribed from the PARK family of genetic mutations has a distinctly different effect on the operation of the neuron in which it is produced.

In order to study how each of these proteins leads to the overall expression of common Parkinson's disease symptoms in a patient, their normal function must be elucidated. Knowledge about the normal functioning of these proteins can be just as important as an understanding of their pathogenic behavior. As research has shown, much of the pathology of Parkinson's disease must be explained not only by the loss of function of a protein or brain region, but also by a gain of function that is detrimental to the normal working of the cell.

The immediate goal of the current research being conducted on Parkinson's disease is to provide a description of how each specific molecular pathway works to affect the Parkinson's disease patient on a macroscopic level. The most prominent questions that today's researchers seek to answer are problems such as these: how does the aggregation of the protein α-synuclein impair the PD patient's cognitive and motor skills? What specific symptoms are made manifest by this molecular abnormality? Parallels will be drawn between each of the causal factors of the disease and their respective expressions in the patient's physiology.

The currently available treatments for Parkinson's disease are continually being accumulated and modified as new research reveals target areas for development. The

disease was originally thought to have been acquired *de novo* due to environmental factors or toxins. However, within the last ten years or so, increasing evidence for the genetic basis of the disease has surfaced. Understanding these mutations and inherited traits better will open countless avenues for treatment, from drug development to gene therapy.

Each of the molecular mechanisms of the disease presents a unique opportunity for management of the symptoms it produces. The experiments described in this review investigate how and where each pathogenic molecular pathway may be intercepted, perhaps with a new drug, that would restore the natural biological process or at least mitigate the effects of the disease. Noting the diversity of Parkinson's disease cases, it is probable that the conclusion of this problem will be a multifaceted one. It is likely that the various mechanisms of the disease, while there will be many parallels, will also differ substantially. Because of this, a multiplicity of treatments will be proposed to fit each distinct case in a uniquely appropriate way.

**Alpha-synuclein and the Pathology of Parkinson's Disease**

**Current Parkinson's Disease Research Techniques**

The biological and chemical mechanisms regarding abnormal protein aggregation in the brain of Parkinson's disease (PD) patients is forefront in the developments of Parkinson's disease research. The molecular basis of the disease and its various causes have been investigated and analyzed, according to the results of previous experiments. Extensive Parkinson's disease research is also conducted at hundreds of other labs and institutions across the world, including the National Institutes of Health (NIH). This research seeks to explain how these mechanisms correlate with the most common treatment pathway of the

disease. Last, the effectiveness of L-DOPA as a treatment for Parkinson's disease treatment will be analyzed.

**Molecular Techniques Reveal the Cellular and Molecular Mechanisms**

Parkinson's disease has a variety of genetic and epigenetic sources. These sources have been discovered and analyzed by a plethora of methods, including NMR, spectrophotometry, genetic techniques, proteomics, neuroproteomics, analysis of enzyme kinetics, and pharmacokinetics. Specific results from selected techniques will be described throughout the discussion in order to analyze further the mechanism of protein aggregation in Parkinson's disease.

One of the most important sources for Parkinson's disease pathology appears to be the abnormal aggregation of a protein called α-synuclein in the brain. Alpha-synuclein has only primary structure, and there is generally no secondary or tertiary structure determined by its amino acid sequence. Many proteins that remain in an unfolded state serve as chaperones in the cell and can aid in the folding of new proteins (Irvine, El-Agnaf, Shankar, & Walsh, 2008). However, NMR spectroscopy has revealed that when α-synuclein is bound to a lipid or micelle surface, the protein will adapt a secondary structure: a right-handed alpha helix interrupted by a single break (Bussell & Eliezer, 2003).

Parkinson's disease is confirmed in large part by the presence of Lewy bodies (LB) in the substantia nigra, which are found in the brains of autopsied PD patients. Lewy bodies occur in several other neurodegenerative diseases as well (Shults, 2006). Alpha-synuclein has been found to be the primary component of LB. This protein is found in presynaptic nerve terminals and associates with the membranes of vesicles in the

synapse  (Irvine et al., 2008). This association is most likely due to the formation of the

right-handed alpha helix structure it forms when associated with lipids.

There are several stages involved in the kinetics of α-synuclein aggregation, and

some of these stages can be altered by nutraceuticals (Mazzio, Close & Soliman, 2011).

The first step is that an α-synuclein monomer must be modified; the second is the

interaction of modified monomers to form small aggregates; the third is the elongation of

these aggregates into fibrils, which are subsequently deposited in neurons and Lewy

bodies (Mazzio, Close & Soliman, 2011).

Spectrophotometry is widely used in the determination of enzyme kinetics related

to Parkinson's disease, especially in relation to the enzyme Tyrosinase. Tyrosinase

catalyzes several different reactions in the body, including the conversion of L-Tyrosine

into L-DOPA (Chang, 2009). L-DOPA is an intermediate in the pathway to dopamine,

and so this enzyme is of great interest to PD research.

In the kinetic analysis of Tyrosinase, varying amounts of enzyme, substrate,

inhibitor, or whichever aspect of the reaction is to be studied, are added to reaction

mixtures (Duckworth & Coleman, 1970). These mixtures are immediately placed in a

spectrophotometer and the absorbencies are read at consistent time intervals. The speed

of the reaction can then be determined from these recorded values by plotting a graph.

A Michaelis-Menten plot is used for an enzyme-catalyzed reaction in order to

determine $V_{max}$, the maximum velocity of the reaction, and $K_M$, the Michaelis constant. In

addition, a Lineweaver-Burk plot can be used to analyze the inhibition of the enzyme

(Duckworth & Coleman, 1970). This is also useful in PD research because Tyrosinase

has a large number of biochemical inhibitors and multiple catalytic activities (Chang, 2009).

**Two Possible Conclusions from the Available Data.**

The primary structure of the α-synuclein protein is tripartite. That is, it consists of three distinct but connected regions: the α-helical N-terminal region, the central region, and the C-terminal region. The α-helical N-terminal region is used to bind hydrophobic lipid membranes (Irvine et al., 2008). Without this region, α-synuclein would not be able to bind to vesicles in the synaptic cleft. Thus, it could be inferred that a mutation in this sequence could cause a loss of function of the α-synuclein gene product.

However, α-synuclein may still be able to aggregate easily if the central region were to remain intact. The central portion of the α-synuclein polypeptide is composed of hydrophobic amino acids that are prone to association with other similarly hydrophobic molecules. On the other hand, the C-terminal region of α-synuclein is made up of many hydrophilic amino acids, largely aspartate and glutamate, including a significant number of prolines. This portion of the α-synuclein polypeptide will naturally tend to have contact with the surrounding cytosol or other charged molecules near it.

Alpha-synuclein aggregates, a primary defining feature of Parkinson's disease, are most often attributed to the action of the hydrophobic central region  (Irvine et al., 2008). Because hydrophobic molecules have no dipole to attract the dipole of water, water is far more attracted to its own molecules, leading to aggregation of these predominately Van der Waals molecules. These large aggregates of α-synuclein then coalesce further into more ordered aggregates known as amyloid fibrils, thereby

decreasing the entropy of these molecules (Bussell & Eliezer, 2003). Fibrils found

purified from Lewy bodies resemble closely the structure of these amyloid fibrils.

As previously mentioned, Lewy bodies containing α-synuclein aggregates and

amyloid fibrils are found in autopsies of the brains of Parkinson's disease patients. It is

interesting to note that dopamine and other similar catecholamines have been found to

stabilize the protofibril stage of α-synuclein aggregation by their specific interactions

(Irvine et al., 2008). Lewy bodies are found predominately in the substantia nigra, and so

a question of cause and effect arises. The substantia nigra is so named because of the dark

pigmented neurons found in that area; this pigment is a by-product of dopamine

synthesis. Therefore, there are two possible conclusions.

**Inferences to Guide the Development of Therapies**

The first possible conclusion is that the neurons of the substantia nigra are more

susceptible to α-synuclein aggregation due to their high level of dopamine production

(Irvine, El-Agnaf, Shankar, & Walsh, 2008). The second conclusion is that the *lack* of

dopamine production by the cells in the substantia nigra leads more directly to the motor

dysfunction symptoms of Parkinson's disease. Another piece of information that may

serve to clear up this issue is related to pharmacokinetics. The most commonly prescribed

and most widely effective drug for PD is L-DOPA, also commonly known as levodopa

(Stocchi, 2006).

L-DOPA, unlike the neurotransmitter dopamine, can cross the blood-brain barrier

(Stocchi, 2006). Once in the brain, it is hydroxylated and converted by the enzyme

tyrosinase into dopamine by a series of steps (Chang, 2009). This drug has been shown to

be effective until about 3-5 years of treatment have gone by (Pienaar, Daniels, & Gotz,

2008). After this initial period, L-DOPA begins "wearing off," and the symptoms of PD

return in force (Pienaar, Daniels, & Gotz, 2008).

  These conclusions, among others, are debated in the scientific community—

especially as to which of the mechanisms researched have the most significant impact on

the genesis, course, and termination of the disease. These controversies are unavoidable

however, due to the multi-faceted nature of Parkinson's disease and the many factors and

molecules involved in each process. The most probable explanation will be an integrated

synthesis of each of the mechanisms proposed, and this will vary from individual to

individual.

**Outlook and Proposed Mechanism**

In light of these factors, one possible inference is that certain cases of Parkinson's disease

begin with an elevation in the level of dopamine being produced in the pars compacta of

the substantia nigra. This increased level of dopamine may lead to increased α-synuclein

aggregation, seeing that dopamine stabilizes the protein's protofibril. This aggregation,

toxic to the cells of the pars compacta, may over time lead to *lack* of dopamine

production—a decrease in the levels of dopamine due to the death of pigmented neurons.

This in turn would lead to the motor dysfunction symptoms characteristic of PD.

  When L-DOPA is prescribed, the artificially increased levels of dopamine in the

brain may mitigate these symptoms for a particular duration of time. However, the

increased dopamine (if not properly regulated) could easily lead to a further increase in

the aggregation of α-synuclein. As the drug continues to affect the PD patient's body and

brain, extensive aggregation may eventually occur, possibly after about 3-5 years. By this

time, the toxic aggregates and amyloid fibrils may have almost completely destroyed the pars compacta.

If this occurs, there would no longer be much free α-synuclein nor cells in the pars compacta capable of regulating the level of dopamine. In this case, L-DOPA would no longer continue to be effective, as seen in the majority of clinical cases. Whether this hypothesis is correct or not remains to be determined. However, it is likely that this mechanism, if found to occur, is only a part of the course of this disease and only in certain cases.

Extensive research remains to be done on the problem of the mechanisms of Parkinson's disease, especially when it comes to the intricacies of protein aggregation. This work will most likely take years and even then a definite conclusion will probably not be found. The mechanism of PD may never be resolved completely due to the high degree of diversity and variability inherent in the disease.

It is clear that new avenues for research need to be explored, specifically in the areas of interaction between the various mechanisms. The solution is most likely to be a balance and conglomeration of several different methods because Parkinson's is very much a multifaceted disease. The more research that is done, especially with the added understanding that there can never be one perfect fix, may not eradicate the problems of the disease but may one day make it a highly survivable and recoverable disease.

**Alpha-synuclein and Dopamine Levels in the Parkinson's Disease Brain**

One of the hallmarks of Parkinson's disease (PD) is the abnormal aggregation of a protein called α-synuclein in the brain (Irvine et al., 2008). Parkinson's disease is confirmed in large part by the presence of Lewy bodies (LB) in the substantia nigra,

which are found in the brains of autopsied PD patients (Shults, 2006). Alpha-synuclein

has been found to be the primary component of LB (Irvine et al., 2008). This protein is

found in presynaptic nerve terminals and associates with the membranes of vesicles in the

synapse (Irvine et al., 2008).

Considering the primary structure of α-synuclein, it is clear that there are three

distinct regions. The N-terminal region is composed of a repetitive sequence of amino

acids, an "α-helical lipid-binding motif of apolipoproteins" (Irvine et al., 2008, pg. 458).

This section is essential for the binding of α-synuclein to vesicles in the synaptic cleft.

The central stretch of the sequence is made up of many very hydrophobic amino acids

(Irvine et al., 2008). In contrast, the C-terminus is composed of predominately

hydrophilic residues.

Alpha-synuclein aggregates may be caused chiefly by problems arising from this

exceedingly hydrophobic central region. These large aggregates of α-synuclein then

coalesce further into more ordered aggregated known as amyloid fibrils. The two articles

discussed in this paper investigate the effects of increased expression of α-synuclein on

neurons, a common cause of the PD pathology.

**Synthesis of Two Related Experiments Examining the Effects of Alpha-synuclein**

**Levels on the Cell**

**Review of Experiment 1: Effect of Alpha-synuclein Levels on Synaptic Vesicle**

**Reclustering.** The first article, "Increased Expression of Alpha-Synuclein Reduces

Neurotransmitter Release by Inhibiting Synaptic Vesicle Reclustering After

Endocytosis," found that over-expression of α-synuclein significantly inhibits the release

of neurotransmitters (Nemani et al., 2010). To elucidate the role of α-synuclein at the

synapse, a special type of imaging technique was used. A fusion of vesicular glutamate

transporter 1 (VGLUT1) to the modified GFP ecliptic pHluorin (VGLUT1-pHluorin)

served to monitor the exo- and endocytosis of synaptic vesicles via the pH changes and

accompanying change in fluorescence of the pHluorin that occur during the release of a

vesicle. VGLUT1-pHluorin was co-transfected with either the wild type human α-

synuclein or the control, an empty vector, into embryonic hippocampal neurons for *in*

*vitro* analysis (Nemani et al., 2010).

This method revealed that the over-expression of α-synuclein inhibits the

exocytosis of synaptic vesicles (Nemani et al., 2010). Electrophysiology experiments in

transgenic mice over-expressing α-synuclein also exhibited inhibition of synaptic

transmission. These mice showed significantly less baseline transmission than wild type,

with results similar to those obtained in culture. The inhibition effect of α-synuclein was

also seen in the rat ventral midbrain, which contains approximately 90% dopamine

neurons (Nemani et al., 2010).

Further imaging using VGLUT1-pHluorin and related imaging techniques

revealed that the over-expression of α-synuclein results in a reduction of the readily

releasable and recycling synaptic vesicle pools. It was also found that this over-

expression affects the reclustering of synaptic vesicles after endocytosis. This evidence

obtained by VGLUT1-pHluorin analysis was confirmed by a GFP-VGLUT1 control

(Nemani et al., 2010).

The imaging of GFP-VGLUT1 provided direct evidence for the problem with

synaptic vesicle reclustering because it showed the entire time course of the reclustering

of synaptic vesicles after endocytosis. This confirmation supports the conclusion of the

article, which was that the increased levels of α-synuclein seen in both sporadic and familial PD reduce the amount of neurotransmitter released by inhibiting the recycling of synaptic vesicles after endocytosis.

**Review of Experiment 2: Effect of Alpha-synuclein Levels on Dopamine Toxicity.**

The second study, "α-Synuclein Overexpression Increases Dopamine Toxicity in BE(2)-M17 Cells" by Bisaglia et al. (2010), explored the interplay between α-synuclein and dopamine. This experiment specifically investigated the possible role of oxidative stress in Parkinson's disease by looking at the redox reactions in nigral dopaminergic neurons. Dopaminergic human neuroblastoma BE(2)-M17 cells were transfected with WT or A30P mutant α-synuclein (Bisaglia et al., 2010). Then, cellular toxicity was measured.

Two methods were used to measure cellular toxicity: lactate dehydrogenase (LDH) activity-based cytotoxicity assay and fluorescence-activated cell sorter (FACS) analysis (Bisaglia et al., 2010). For the lactate dehydrogenase assay, cells were plated into wells with growth medium and supplemented with dopamine (DA). Catalase was used as a control to remove any hydrogen peroxide produced by extracellular catecholamine oxidation. This control was necessary in order to study only the intracellular effects of DA oxidation. Once the lactate dehydrogenase assay was performed, the percentage of cell death was calculated by the ratio of the activity of LDH in the supernatant to the total LDH activity (Bisaglia et al., 2010).

The expression levels of both types of α-synuclein were about 6 to 8 times higher than the control cells, indicating a level most likely slightly higher than that seen in most PD cases (Bisaglia et al., 2010). The results showed that DA induced cell damage in all cell lines, and that cells overexpressing either the mutant or WT α-synuclein were more

susceptible to the dopamine-induced toxicity (Bisaglia et al., 2010). Fluorescence-activated cell sorter analysis was performed to confirm that the overexpression of α-synuclein increased cell vulnerability to DA by sorting the viable cells from the necrotic and apoptotic cells. These results confirmed those of the lactate dehydrogenase assay (Bisaglia et al., 2010).

Previous studies have shown that oxidized DA can covalently modify α-synuclein, stabilizing its protofibril form. The main results of this study were that over-expression of both WT and mutant α-synuclein produce toxic effects on the cell and that α-synuclein and DA are more toxic together than the sum of their individual toxicities (shown by graphed FACS results). It is believed that this synergistic effect is due to the modification of α-synuclein by oxidized DA in the cytosol (Bisaglia et al., 2010).

There are many issues left unclear at the conclusion of this experiment. For one, the title of this article claims that "α-synuclein overexpression increases dopamine toxicity." However, the conclusion claims that "DA exposure increases the toxicity of the PD-related protein α-synuclein." Does α-synuclein increase dopamine toxicity or does DA increase α-synuclein toxicity? The conclusion avoids this question by calling this a "synergistic toxic effect" (Bisaglia et al., 2010, pg. 5).

It is clear from the results of this and other experiments that DA and α-synuclein can together produce a toxic effect. Although this is true, some of the statements that were made in this article regarding cause and effect most likely need further investigation. In other words, the direct or indirect pathway by which α-synuclein increases the toxicity of DA (or vice versa) has not been made clear by this experiment, and still remains to be determined.

**Contributing factors have a synergistic effect on cytotoxicity.**

Collectively, the two articles discussed in this paper show the detrimental effects of the over-expression of α-synuclein on the brain. These findings are relevant to the current research in Parkinson's disease because α-synuclein is commonly over-expressed due to genetic defects causing multiple gene copies, as previously mentioned. In the first article, it was found that an increased level of α-synuclein in the cell reduces the amount of neurotransmitter released by inhibiting the recycling of synaptic vesicles after endocytosis. In the second article, the synergistic toxicity of α-synuclein and dopamine was explored and quantified.

These articles may together contribute to the elucidation of the PD pathology in several ways. The most important of these relates to the level of intracellular dopamine in the substantia nigra neurons. Since we know from the first experiment that the vesicle recycling after endocytosis is inhibited in the presence of over-expressed levels of α-synuclein, it may be inferred that the levels of dopamine in these cells is increased. Since the cells of the substantia nigra normally release the neurotransmitter dopamine into the synaptic cleft of their target cells, the impairment of these cells in releasing DA might cause elevated levels inside of the neuron.

There may be some mechanism by which the cell compensates for this abnormal increase in dopamine caused by the decreased synaptic vesicle recycling. However, if not, this DA increase would certainly be consistent with the results obtained in the second article. In that experiment, each cell line was treated with increasing concentrations of DA, and then the extent of cellular toxicity was determined. As the concentration of DA used was increased, the level of cell death also increased accordingly. As expected, the

cells expressing both WT and mutant α-synuclein were more susceptible to cell death with this induced dopamine increase.

The results of both of these articles suggest a mechanism by which the over-expression of α-synuclein in the Parkinson's disease brain could lead to neuronal death. There are several contributing factors involved in this mechanism, most of which have a synergistic effect on cytotoxicity. First of all, the hydrophobic central region of the α-synuclein protein contributes to the propensity of these monomers to aggregate. Further, oxidized dopamine is known to stabilize the protofibril stage of this protein's fibrillation. Thus, an increase in the expression of α-synuclein adds to this problem by increasing the propensity of these monomers to aggregate and augments their cytotoxicity.

With more α-synuclein aggregates, less dopamine is released from vesicles into the synaptic cleft. This may cause a build-up of dopamine in the cell, surpassing normal levels even for the dopaminergic neurons. More dopamine causes more stabilization of the α-synuclein protofibril, which in turn leads to further aggregation. This cyclic effect, if shown to be valid, would explain in part why the L-DOPA treatment is ultimately ineffective in PD patients.

In order to validate this hypothesis, the levels of intracellular dopamine would have to be monitored in dopaminergic cells like those of the substantia nigra. Similar experimental setup, like that of the second experiment, would be useful. However, one change would be that the cells should be tested with some type of fluorescently detectable dopamine. If the level of dopamine inside the cells could be visualized by a using a fluorescent tag, the amounts could be quantified and compared between samples.

The experimental setup of the second experiment would be useful if the same

variable and controls were used. The WT and A30P mutant α-synuclein variants along with the same increasing levels of dopamine could be employed. After each cell line was treated with the specific amount of dopamine being used in that trial, the cells could be visualized using fluorescence microscopy. Then, stereology could be used to quantify the amount of fluorescence seen in each sample.

**Impact of synthesis and prospects for further experimentation.** Both of these experiments have broadened knowledge about the interconnected pathways responsible in PD pathology. The suggested experiment should help to clarify the question of whether α-synuclein causes increased intracellular levels of dopamine. It will also assist in the understanding of this disease by authenticating the hypothesis that high levels of dopamine inside these cells contributes to cell death, as this could be measured using the same techniques as in the second experiment discussed. Overall, together these experiments will further aid in the dissection of cellular-level PD pathology as it relates to the over-expression of the α-synuclein gene.

## The Development of Potential Therapies for Parkinson's Disease

**Approaches to Parkinson's disease therapy**

There are many approaches to Parkinson's disease therapies, including both neuroprotective and neurorestorative therapies. Neuroprotective therapies mainly seek to prevent the spread of pathogenesis to new neurons and brain regions. On the other hand, the goal of neurorestorative therapies is to return diseased neurons to their healthy processes. Some neurorestorative therapies also seek to replace the cells that have already died.

At this time, when dopamine prescription is in widespread use for the treatment of Parkinson's Disease, the symptoms of the disease that are unrelated to neurodegeneration

in the midbrain have become most prominent. Symptoms such as depression, dementia, and cognitive decline have the strongest effects on patient mortality. Furthermore, it is these symptoms that arguably have the most poignant effect on a patient's quality of life (Sen & West, 2009).

However, it is important to note that these different categories of symptoms are intricately and inextricably related to one another. Although different approaches will be needed to target each system, there is much evidence for the effectiveness of therapies that try to attack both categories at once. For instance, recent research has shown that both Parkinson's Disease and depression may be mediated by the same mechanism. They have both been linked to the degeneration of the dopaminergic system. Because of this, the possible therapeutic effects of dopamine agonists are being studied in the treatment of depression in PD patients (Lemke, 2008).

**Targeting of Reactive Oxygen Species Involved in Pathogenesis**

Another target being heavily researched is related to the production of reactive oxygen species, which are produced as toxic byproducts in the dopaminergic neurons. These reactive oxygen species can be formed both inside and outside the mitochondria (Zhou, Huang, & Przedborski, 2008). If they are not degraded and disposed of properly, these byproducts can be very dangerous to neurons and other cells because they will begin to react spontaneously with important compounds in the cell. If this process is not stopped, it will almost inevitably lead to apoptosis.

Clinical trials are currently being conducted in order to determine the mechanisms by which this oxidative stress can occur and the possible ways it can be limited. One promising compound for this task is Coenzyme Q10. Coenzyme Q10 helps to balance and enhance mitochondrial electron transport and ultimately ATP synthesis. This may

allow the mitochondria to function more competently and reduce the production of

dangerous reactive oxygen species (Sen & West, 2009).

**Regulating Intracellular Calcium Levels**

The adverse effects of reactive oxygen species in the brain can also be reduced by

other means. Molecules such as vitamin E, a natural antioxidant, and selenium have been

tested for their effectiveness at neutralizing the reactive oxygen species. More

specifically, L-type $Ca^{2+}$ channels have been shown to be important in the long-term

survival of dopaminergic neurons. These channels help to regulate the activity of the

neurons by keeping the concentrations of calcium at a low level inside the cell. The

endoplasmic reticulum and mitochondria both play a role in this process as well. ATP is

used to pump calcium out of the cytosol and into these compartments.

Additionally, calcium channel blockers are being used in clinical trials. The

reason for this is to ameliorate the mitochondrial dysfunction seen in the substantia nigra

of the Parkinson's Disease brain. If the mitochondria becomes damaged and is not

functioning properly, as previously described, the L-type $Ca^{2+}$ channels may not be

regulating the levels of intracellular calcium any longer. This would lead to an increase in

intracellular calcium inside of these neurons.

An abnormally high level of intracellular calcium is known to cause apoptosis by

signaling the release of the electron transport protein Cytochrome C from the

mitochondria. The presence of this mitochondrial protein, Cytochrome C, in the cytosol

begins a cascade of protein interactions inside the cell that terminates in apoptosis

(Andreyev & Fiskum, 1999). Because of this, the need for the functioning of these

calcium channels may play a significant part in the Parkinson's Disease pathology.

Much ATP is needed for these processes, and so the level of oxidative

phosphorylation occurring in these cells is quite high. This is characteristic of the

dopamine-producing neurons. Thus, along with the generation of reactive oxygen

species, it is clear that this high level of oxidative stress may shorten the lives of these

cells. Mitochondrial DNA damage is also an important factor in this aging process,

because its DNA can easily be damaged by the nearby oxygen species (Simunovic et al.,

2009).

**Targeting N-methyl-D-aspartate Receptor Networks**

A role for *N-methyl-D-aspartate* (NMDA) receptors, a type of glutamate receptor,

has also been implicated. The ion channel that is controlled by the NMDA receptor is

both neurotransmitter-dependent and voltage-dependent. This type of ion channel

requires a specific chemical neurotransmitter as well as a specific threshold voltage in

order to work. Magnesium ions ($Mg^{2+}$) normally block this ion channel when the

postsynaptic membrane is at its resting potential. This blockage prevents calcium ions

($Ca^{2+}$) from entering through the channel. These ions, because they are polar, are unable

to pass through the hydrophobic lipid bilayer membrane of the cell (Carlson, 2011).

However, once the membrane of the postsynaptic cell becomes depolarized, as the

result of a passing action potential travelling down the membrane, the magnesium ion is

displaced. Glutamate then attaches to its binding site, which was previously blocked by

magnesium, and the ion channel opens so that calcium ions can enter the dendritic spine

(Carlson, 2011).

There is evidence that NMDA receptors promote the survival of the nigrostriatal

dopaminergic neurons. Although the exact mechanism for this is not known, it has been

supported by several experiments. The blocking of these receptors using non-competitive

antagonists substantially reduced the survival of dopaminergic neurons in one experiment. In further support of this hypothesis, when high levels of $K^+$ were induced in these same neurons, the L-type $Ca^{2+}$ channels were activated (Michel et al., 2007). This experiment suggests that the blockading of NMDA receptors via this molecule prevents the entry of calcium ions through the receptor pore (Katsuki et al. 2003).

These observations, along with the pervious research, led to the conclusion that NMDA receptors control the firing of the dopaminergic neurons. These receptors regulate both the spontaneous and NMDA-induced firing. The complex integration of these neurotransmitter receptors and ion channels illustrates the significance of electrical activity on the survival or the nigrostriatal dopaminergic neurons. Not only is this delicate balance essential to development but it is also vital to the functioning of the adult brain. Thus, changes in the electrical currents inside of the brain of a Parkinson's disease patient may contribute to the cell death of the substantia nigra neurons (Michel et al., 2007).

The examination of these molecular networks is difficult in that each of the neurotransmitter systems is connected to another. For instance, serotonergic neurons can aid in process of L-DOPA induced dyskinesia by causing an imbalance in the transmission of dopamine (Navailles & De Deurwaerdere, 2012). Additionally, the neurons of the subthalamic nucleus contain glutamate, which also interacts with the glutamate receptor NMDA (Shen & Johnson, 2010).

**The Potential of Neurosurgery**

A common neurosurgical option for patients with Parkinson's disease is deep brain stimulation. In the past, neurosurgical procedures have been performed on the subthalamic nucleus, thalamus, or globus pallidus pars interna. Recent developments have led to the preferential use of deep brain stimulation of the subthalamic nucleus. The

subthalamic nucleus and globus pallidus pars interna have increased neuronal activity in Parkinson's Disease. (Williams et al., 2010).

These specific brain regions are believed to be responsible directly for motor dysfunction symptoms. It was been shown that monkeys with induced parkinsonism can be given improved motor function if lesions are made in these brain regions. Lesions can also be made in the brains of Parkinson's disease patients with positive effects on motor function. However, there can be a significant amount of damage occurring as side effects as well. Fortunately, deep brain stimulation is capable of producing effects very similar to that of a lesion except without the disadvantage of destroying brain tissue (Williams et al., 2010).

### Expectations for Further Research

The complex nature of Parkinson's disease requires that the conclusion of this research, if one is ultimately reached, will be equally complex. Given the multifaceted nature of the disease and the various primary causes that have been identified, it is clear that treatment options will need to be tailored to the individual case. Many different mechanisms have been elucidated as time continues, and each of these opens the door to another possibility for a treatment option. The more treatment options that can be made available to patients, the more promising the results.

Overall, the laboratory and clinical research being conducted has tended towards that end, and can be expected to continue. Most importantly, although the goal of this research is to find common pathways and disease processes which can be targeted by medicine, the ultimate purpose is to heal the individual patient. The multidimensional aspects of Parkinson's disease reflect diversity in the patients it affects, and the most successful research will take that characteristic into account.

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