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A Possible Mechanism for the Propagation of Pathological Proteins in Parkinson's Disease

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Abstract

Parkinson's disease is a common neurodegenerative disorder which recently has come to be regarded as a prion-like foldopathy, the latter term implying that the intercellular trafficking of abnormally folded proteins transmits disease between cells and produces pathology in target cells through the induction of a mis-folded state in normal protein in those cells. In the case of Parkinson's disease, the prion-like protein primarily involved is alpha-synuclein and inclusions of this protein called Lewy Bodies are commonly found in the substantia nigra of affected patients. Over recent years considerable evidence has been developed to show that a variety of extracellular signaling molecules called intracrines can act in the intracellular space of their cells of synthesis or in target cells after intercellular trafficking. Thus there is some commonality between intracrinephysiology and the pathophysiology of prion-like foldopathies such as Parkinson's disease. Here these issues and a possible nexus between Parkinson's disease and intracrine biology are discussed.

Abbreviations

MPTP: 1-methyl-4-phenyl-1,2,3,6-terahydropyridine; DMT: Divalent Metal Transporter; 6-OHDA: 6-Hydroxydopamine; IRP: Iron-Responsive Element Binding-Protein or Iron Regulatory Protein; IRE: Iron Response Element; 3'-UTR: 3'-Untranslated Region; 5'-UTR: 5'-Untranslated Region; NO: Nitric Oxide

Background

Parkinson's disease (PD) is a common, progressive, degenerative neurological disease clinically characterized by resting tremor, rigidity, bradykinesia, masked facies, changes in posture, and in time, dementia. Pathological changes center on the substantia nigra where a drop out of dopaminergic neurons is seen. Frequently inclusions known as Lewy bodies (LB), consisting of the protein alpha synuclein, iron and other moieties, are present in neuronal cell bodies. Other neurons contain alpha-synuclein filaments. PD occurs in both heritable and sporadic forms; at least 18 genes are known to be linked to the disorder. The augmentation of dopaminergic stimuli through the provision of compounds such as L-DOPA partially replaces the loss of dopaminergic neurons and provides symptomatic relief. It is unclear, however, if this therapy slows the progression of the disease to any significant extent. Of note, transplantation of dopamine-producing neurons into substantia nigra leads to symptomatic relief, but tellingly, after some years benefit lessens and Lewy bodies are found in degenerating transplanted cells. This suggests a non-cell autonomous pathological process [1-5].

Over the last decade, new insights into the nature of many neurodegenerative disorders have been developed. These have arisen from the demonstration that Creutzfeld-Jacob disease (CJD), scrapie, kuru, mad cow disease, and related disorders result from infection with proteinaceous infectious particles, or prions, which although apparently containing no nucleic acid can infect normal cells and propagate by inducing pathological, infectious conformational changes in their normal protein homologues. Thus the ingestion of infected tissue can introduce infectious prions to an individual and these prions can then spread to the brain where they propagate through neural tissue and produce neurodegeneration. Subsequently, it became apparent that other neurodegenerative disorders while not strictly infectious in terms of easy transfer between infected persons or animals, display characteristics of prion biology in that they appear to spread through neural tissue. Included among these disorders are Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease. In Alzheimer's disease, the predominant, but often not the sole, prion-like proteins appear to be beta-amyloid and tau, in amyotrophic lateral sclerosis, SOD-1 or TD43, and in the case of Parkinson's disease, alpha-synuclein. The spread of infectivity in Parkinson's disease from patient brain to transplanted cells is consistent with this idea of prion-like spread in PD [6-12].

At the same time, considerable progress has been made in defining the actions and biology of so-called *intracrines*---peptide extracellular signaling moieties that serve intracellular functions either in their cells of synthesis or in target cells after internalization. Intracrine functionality is associated with a wide variety of peptides/proteins such as hormones, cytokines, growth factors, enzymes, and DNA binding proteins among others. General principles of intracrine biology have been developed and among these is the notion that intracrines often form feed-forward, or positive feedback, loops in cells. This has the effect of producing persistent changes in cells and produces a form of differentiation [13-16]. At first glance there are crude but definite similarities between the actions of prions and prion-like proteins and intracrines. This has led us to explore these possible relationships and to propose that intracrine amplification, as occurs during the formation of intracrine feed-forward loops, is a characteristic of many common neurodegenerative disorders. However, to date, there is no conclusive evidence for the obligatory amplification of prions or prion-like proteins in neurodegenerative diseases. Indeed, the available evidence suggests that if amplification of prion-like proteins occurs, it does so only transiently at the onset of cellular infection accumulated abnormal protein moieties

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thereafter being sufficiently abundant to propagate in infected cells demonstrating no up-regulation of normal protein isoforms. It is with this in mind that we turn to Parkinson's disease and discuss a possible nexus between it and intracrine biology [13-16].

An Intracrine View of Parkinson's Disease

In general, intracrine peptides subserve normal physiologic functions both as external signaling molecules (as, for example, after secretion) and as regulators of intracellular functions. Thus, the notion that these entities function in normal physiology, and not only in disease, is integral to typical intracrine functionality. Similarly, intercellular trafficking of these peptides/proteins either after secretion, atypical secretion, cell death, release in exosomes, or passage through intercellular channels called nanotubes---followed by internalization by target cells--- are features of intracrine physiology. And finally, feed-forward loops, often involving amplification of the intracrine itself or of elements of its signaling cascade, are characteristic of intracrine differentiation [13-16]. It is noteworthy that prions and prion-like entities share many of these features. For example, prions, in their normal configuration before conversion to pathological configurations, have been reported to have physiological functions in regulation of synaptic plasticity and possibly in normal development and adult neural functioning. Such functions have been suggested for the native forms of beta-amyloid, SOD-1, TD43, and alpha-synuclein [10,11,17-21]. Moreover, there is evidence supporting intercellular trafficking, by one or another of the mechanisms employed by intracrines, for prions, beta-amyloid, amyloid precursor protein (the precursor of beta-amyloid), tau, TD43, SOD-1, and alpha-synuclein [6-8,22-24]. Thus, there is evidence to support the notion that these conditions, and likely other chronic neurodegenerative disorders such as traumatic encephalopathy, are intracrine in nature. In the case of alpha-synuclein, it has been shown that the addition of the protein to neuronal cultures stimulates early neurite outgrowth that is dependent on synuclein internalization, with internalized alpha-synuclein found in cytoplasm and to a lesser extent in nucleus [21]. These findings, coupled with the established ability of the protein to traffick between cells, indicate that alpha-synuclein in its native form acts in an intracrine fashion and its intracrine regulation of cell growth suggests a physiologic role for the protein in addition to the microtubule stabilization, regulation of vesicle trafficking and dopamine release, modification of synaptic structure, and other functions that have been suggested for it [18-21]. It is the issue of feed-forward loops, or more generally, of amplification, that is problematic for ascribing more robust intracrine functionality to prion-like proteins, including alpha-synuclein, or to their mis-folded forms.

There is, to be sure, evidence to suggest the existence of amplification in neurodegenerative disorders, but none is conclusive. For example, TD43 aggregates are cardinal features of many forms of amyotrophic lateral sclerosis and TD43 represses the translation of its own RNA. Hence, the sequestration of TD43 in aggregates likely increases the translation of its message and therefore its own production-a feed-forward loop [10,11,13]. Similarly, prion disease is most easily spread to transgenic animals that over-express normal prion protein, suggesting that up-regulation of prion protein is important in the disease process [13]. Other examples can be given but in totality, while there is evidence to suggest a role for amplification in the pathogenesis of neurodegenerative disorders, there is no conclusive evidence for its necessity. A similar, but distinct situation, pertains in the case of Parkinson's disease. Here the preponderance of evidence points to the up-regulation of alpha-synuclein in the substantia nigra as an initiator of the disease [25-39]. Over expression of alpha-synuclein secondary to gene multiplication is associated with heritable forms of Parkinson's disease [3,37]. Some studies have demonstrated increased brain alpha-synuclein mRNA in sporadic Parkinson's disease substantia nigra, although others have failed to do so [25-37]. Alpha-synuclein mRNA is increased in the substantia nigra dopaminergic neurons in an acute model of Parkinson's disease employing the neurotoxin MPTP [25]. Analysis of substantia nigra DNA from patients with sporadic Parkinson's disease has demonstrated altered alpha-synuclein gene methylation consistent with enhanced gene expression [33]. The routine use of transgenic animals over-expressing alpha-synuclein as vehicles for propagating disease after intracerebral seeding with aberrantly configured alpha-synuclein is consistent with a role for alpha-synuclein in disease propagation [9]. On the other hand, there is data suggesting that alpha-synuclein expression is down-regulated in surviving substantia nigrans neurons in established Parkinson's disease. Mechanistically, these data collectively suggest that the initial accumulation of aggregated alpha-synuclein early in the infection of a cell is the result of increased alpha-synuclein production and is not simply the result of ongoing protein sequestration, while the role, if any, of up-regulation later in the course of cell pathology is unclear [31,38]. Parkinson's disease differs from other neurodegenerative diseases in that the available evidence makes up-regulation of the involved prion-like protein during disease initiation highly likely. However, up-regulation---transient or permanent---of the involved prion-like protein does not prove amplification is occurring. Up-regulation could be needed simply to increase the probability that an initial aberrant conformation of alpha-synuclein is produced (either spontaneously or after seeding), resulting in subsequent disease progression that is supported solely by the normal expression of the native protein. For intracrine-like amplification, it must be shown that the prion-like protein is involved in triggering that up-regulation and subsequent disease propagation. This then focuses attention of the regulation of alpha-synuclein synthesis.

How might alpha-synuclein be up-regulated in Parkinson's disease? It has been suggested that in neurodegenerative disorders such as amyotrophic lateral sclerosis, abnormal RNA binding protein/RNA interactions lead to disordered protein synthesis and disease [10,11]. While this kind of process is possible in Parkinson's disease, there is little evidence to support it at this time. The transcription factor GATA2 is expressed in the substantia nigra and up-regulates alpha-synuclein [38]. Parenthetically, the related transcription factor GATA1 up-regulates alpha-synuclein and several enzymes involved in heme metabolism in cells of erythroid lineage, thereby suggesting, however remotely, a possible link between alpha-synuclein and heme biology. But GATA1 is not expressed in the substabtia nigra and there is no evidence that alpha-synuclein can up-regulate GATA2 and thereby establish a feed-forward loop [38]. Similarly, the neuroprotein pentraxin II is involved in neuroplasticity and is up-regulated in a sustained fashion in Parkinson's disease. Along with alpha-synuclein, it is expressed in substantia nigra neurons and is a component of Lewy bodies [39]. However, as in the case of GATA2, there is no evidence to

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support the formation of a feed-forward loop with alpha-synuclein, although in the case of both proteins such evidence should be sought. Other possible regulators of alpha-synuclein and participants in a feed-forward mechanism could be suggested and, indeed, epigenetic causes of amplification are supported by the observation of hypomethylation of the alpha-synuclein gene in Parkinson's disease consistent with enhanced transcription [28,33,34]. Also, the presence of normal alpha-synuclein in nucleus raises the possibility that its replacement by misfolded alpha-synuclein (either randomly generated or internalized by the cell) could directly, or indirectly through effects on heavy metal binding, up-regulate alpha-synuclein gene transcription [16,19]. But taking all the available evidence into consideration, the most likely candidate to regulate any amplification appears to be iron.

The concentration of iron in the substantia nigra is elevated in Parkinson's disease, and while much of this iron is to be found in microglia and astrocytes, the iron concentration of the dopaminergic neurons is also increased [40-43]. The 5' untranslated region of alpha-synuclein mRNA-like the mRNA of several other proteins involved with iron metabolism-contains a functional iron response element (IRE) to which iron regulatory proteins (IRPs) bind. Binding of iron to iron regulatory protein 1 (IRP1) reduces its binding to the IRE and enhances alpha-synuclein message translation and synthesis; similarly, IRP2 is degraded in the presence of iron and its binding to IREs is therefore also reduced in iron replete states [44-46]. An *in vitro* study has shown that iron chelation reduced alpha-synuclein translation indicating a role for iron in the regulation of synuclein expression, but excess iron did not up-regulate translation in this model [45]. These studies were conducted in embryonic kidney cells, not neurons. It remains probable that the alpha-synuclein IRE can produce enhanced translation in neurons after iron loading as would be expected---for example, as it does in the case of ferritin. This is likely because knock down of IRP1 in a neuronal cell line up-regulates synuclein. Moreover, additional studies show that iron and/or iron-mediated oxidative stress up-regulates alpha-synuclein, further supporting a role for iron in alpha-synuclein up-regulation [46-49]. Cellular iron content is elevated in the substantia nigra in Parkinson's disease and, indeed, iron is a component of Lewy bodies. Thus, if abnormal or up-regulated alpha-synuclein were to increase cellular iron, the opportunity for a feed-forward iron-synuclein loop would exist. The fact that like other iron-regulated proteins, alpha-synuclein mRNA contains an IRE suggests that it is involved in iron metabolism. Recently it has been shown that alpha-synuclein is a ferri reductase capable of reducing Fe^{+++} to Fe^{++}. This enzymatic activity potentially increases the level of bio-available iron and oxidative stress in the cell, but whether this facilitates a feed-forward loop is unknown [50]. On the other hand, it has also been shown that amyloid precursor protein (APP), the substrate for the production of beta-amyloid in Alzheimer's disease, is an important regulator of cellular iron. APP regulates iron extrusion from cells by stabilizing cell surface ferrous iron exporter ferroportin. Zinc overload results in tau phosphorylation and secondarily to decreased trafficking of APP to the cell surface resulting in increased cellular iron [40-58]. Phosphorylated tau is, in fact, found in Parkinson's disease, as in Alzheimer's disease, the result of increased tissue zinc, or other causes [58]. Thus, an interaction between alpha-synuclein---in native, oligomeric, or aggregated form---with zinc metabolism could provide a mechanism for closing a feed-forward loop. A clue to how this could occur is found in the example of the function of the gene *PARK9*.

Mutations in *PARK9* are associated with early-onset Parkinson's disease. In cell culture the PARK9 gene product reduces alpha-synuclein, apparently by pumping zinc into vesicles and thereby secondarily enhancing (a) autophagy of alpha-synuclein and (b) its externalization in exosomes. In some models, up-regulation of *PARK9* reduces alpha-synuclein toxicity Knock-down of *PARK9* is associated with zinc over load. PARK9 protein levels are reduced in Parkinson's disease while mRNA levels are elevated suggesting PARK9 dysregulation/dysfunction [59-64]. Thus, a schema for the pathogenesis of Parkinson's disease in the face of *PARK9* dysfunction could involve increased cellular zinc resulting in decreased APP-mediated cellular iron extrusion (secondary to decreased APP trafficking to cell surface as the result of zinc-induced tau phosphorylation) followed by increased intracellular iron with iron-mediated alpha-synuclein up-regulation that results in a supply of the protein for spontaneous misfolding or misfolding secondary to the internalization of a seed of misfolded alpha-synuclein; moreover, iron itself induces alpha-synuclein aggregation [40,45-51,54,56-64]. Decreased alpha-synuclein autophagy secondary to *PARK9* dysfunction could further increase cytoplasmic alpha-synuclein levels, increase the probability of protein mis-folding and therefore increase disease severity and spread. This schema could serve as the platform for the development of a general mechanism of Parkinson's disease pathogenesis if an appropriate nexus between one or another alpha-synuclein moiety and either *PARK9* function or zinc homeostasis exists. For example, there is evidence to indicate that alpha-synuclein aggregation is associated with the aggregation of PARK9 protein either in Lewy bodies or more frequently in cytoplasmic aggregations, and un-denatured PARK9 protein is reduced in Parkinson's disease cortex [62,63]. If this is correct, aggregation could lead to diminished PARK9 function in Parkinson's disease and provide a mechanism for closing an alpha-synuclein feed-forward loop that results in progressive disease. The observation that surviving dopaminergic neurons in Parkinson's disease substantia nigra contain elevated levels of PARK9 mRNA is consistent with decreased native protein levels as the result of aggregation or degradation, followed by compensatory gene up-regulation, although blocked message translation could be occurring. But in any case, diminished PARK9 functionality would complete an alpha-synuclein feed-forward loop. In addition, there is evidence that once oxidative stress is produced (in particular during high iron conditions), IRP1, and likely IRP2 as well, paradoxically are up-regulated leading to down-regulation of ferroportin. Ferroportin, which exports iron from cells, is normally up-regulated in the face of elevated intracellular iron because of decreased IRP binding to its mRNA IRE, but it is down-regulated during oxidative stress with subsequent cellular retention of iron and exacerbation of oxidative stress [65-76]. This positive feedback loop, which has been demonstrated in cell culture, could then also serve to augment intracellular iron content and cellular damage [70,71]. However, because up-regulation of iron regulatory proteins 1 and 2 (IRP1, IRP2) in the face of elevated intracellular iron would be expected to down-regulate alpha-synuclein translation, it is unlikely that this mechanism is operative in the dopaminergic neurons of the substantia nigra during the period of obligatory alpha-synuclein amplification that is postulated here. Later in the progression of intracellular

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pathology, this mechanism could play a role in the induction of cell death in these neurons. It must be recalled that it is here assumed that alpha-synuclein amplification is necessary only in the initiation of a cell's infection; after significant aggregation has occurred, continued aggregation can occur on the background of normal or low synthesis of the protein. In this context, down-regulation of alpha-synuclein, by up-regulation of IRPs or other mechanisms, late in the infection of a cell would not be surprising [13,31,37].

Although the mechanisms mentioned above are plausible, they may not be complete because it has been shown that large scale dysregulation of iron metabolism occurs in Parkinson's disease and not simply because of decreasing APP trafficking or relative down regulation of ferroportin. In fact, lactoferrin and lactoferrin receptor (a system comparable to the transferrin/transferrin receptor system for importing iron into cells) is up-regulated in melanized dopaminergic neurons in Parkinson's disease. Similar results were found in an MPTP model of Parkinson's disease. Also, DMT1 (+IRE)-one of the divalent metal transporter 1 subtypes that contains an IRE and transports internalized iron from endosomes to cytoplasm-is also increased in Parkinson's disease substantia nigra as well as in some models of the disease. On the other hand, transferrin receptors, which might have been thought to be down-regulated in high iron conditions, are unchanged in Parkinson's substantia nigra. IRP1 binding to ferritin mRNA is not reduced in Parkinson's disease substantia nigra in spite of elevated tissue iron. In a 6-OHDA model of Parkinson's disease ferroportin is down-regulated in the face of increased cellular iron secondary to up-regulation of DMT 1 and 2. All of these changes serve to increase intracellular iron. They, along with other findings, suggest a major disorder of iron metabolism in Parkinson's disease. Oxidative stress up-regulation of IRP1 could explain stabilization of DMT1 (+IRE) mRNA and increased synthesis of the protein. DMT1 (+IRE) mRNA, like ferroportin and transferrin receptor mRNA, contains an IRE in its 3'UTR; binding of IRP to this site stabilizes the messages and increases translation. The same process therefore could explain the failure to down-regulate transferrin receptors and ferroportin in Parkinson's disease. It could also explain the normal degree of IRP1 binding to ferritin mRNA (which because the ferritin mRNA IRE is in the 5' UTR, prevents up-regulation of the protein). How the alterations of lactoferrin/lactoferrin receptor are produced remains unclear but they presumably are not related to disordered IRP function [65-76]. A possible explanation for more global iron dysregulation in Parkinson's disease is disordered microRNA (miRNA) function. miRNAs are regulatory RNAs that undergo processing, first in the nucleus, and then in the cytoplasm. It has been suggested that the cytoplasmic processing of these moieties by the DICER complex is inhibited by iron. Because among miRNA targets are proteins involved in iron metabolism, a blunting in miRNA processing could produce the transferrin receptor, lactoferrin/lactoferrin receptor, DMT1, and ferritin results noted above. It would be expected to also increase ferroportin activity, but other mechanisms such as oxidative stress and up-regulation of DMTs could off-set any such change. In a similar fashion, heme facilitates miRNA nuclear processing by the Drosha/DGCR8 complex through enhancing DGCR dimerization; if oxidative stress sufficiently up-regulated heme oxygenase1 (known to be elevated in the vicinity of Lewy bodies), the resulting decrease in nuclear heme could also impair miRNA processing with secondary effects on intracellular iron [77-80]. It appears that there exist multiple levels at which elevated cellular iron, once it occurs, could feedback to produce persistent iron dysregulation. More generally, the point to be made here is not the identification of any specific mechanism for the up-regulation of iron or alpha-synuclein, but rather the plausibility and potential importance of such mechanisms.

The potential mechanisms by which pathology is produced by any quasi- intracrine functionality of alpha-synuclein are unknown and a wide variety of possibilities have been suggested in addition to any pathology produced by iron-mediated oxidative stress. Because these proposed mechanisms apparently differ from the normal functionalities of alpha-synuclein they are properly to be considered epiphenomena rather than intracrine in nature. One proposal is that enhanced oxidative stress by any of a variety of mechanisms could play a role in the production of pathology. If so, the loss of effective alpha-synuclein secondary to oligomerization could relieve its normal down-regulation of tyrosine hydroxylase, and therefore of dopamine production, in substantia nigra. Dopamine overproduction would be expected to lead to augmented oxidative stress and pathology. This scenario could explain the selected loss of dopaminergic neurons in Parkinson's disease [1,81]. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are a frequently encountered cause of familial Parkinson's disease. LRRK2 function is associated with the regulation of neuroinflammation in models of alpha-synuclein overexpression. This raises the possibility that maladaptive inflammatory responses could contribute to pathology in Parkinson's disease. However, because LRRK2 trafficks widely in exosomes and is multifunctional (for example, it facilitates formation of alpha-synuclein inclusions), it may enhance cell death in other ways [82,83]. The possibility that LRRK2 mutants increase alpha-synuclein trafficking in exosomes and thereby enhances pathology should also be considered.

Implications

The implications of these suggestions are both theoretical and practical. Collectively, the available data suggests that elements of intracrine physiology are operative in Parkinson's disease. First, native alpha-synuclein itself is an intracrine: it acts in the intracellular space at microtubules, nucleus, and in association with mitochondria; it trafficks between cells (actually in a regulated fashion); it is internalized by target cells after which it is active; it has physiologic functions on target cells. Second, like normal-alpha-synuclein, mutant and aggregated forms of alpha-synuclein traffick between cells and form aggregates in target cells. Third, aggregates, via one mechanism or another, could lead to up-regulation of alpha-synuclein permitting development of intracellular disease and propagation to nearby cells----further evidence of such alpha-synuclein amplification should be sought. These characteristics are all similar to those of established intracrines. On the other hand, the intracrine-like action seen in Parkinson's disease and other common prion-like neurodegenerative diseases differs from classical intracrine function. First, in the classical case the intracrine moiety undergoing amplification is the normal intracrine-it is in Parkinson's disease as well but this serves mainly to permit the up-regulation of an abnormal form of the intracrine. To put this another way, while the normal intracrine is the major factor in classical intracrine action, in Parkinson's disease and other neurodegenerative diseases (patho)physiological action resides in an abnormal form of the normal protein produced after its

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up-regulation. Also the mechanisms of normal (intracrine) prion-like protein amplification are not necessarily the same as any pathological amplification that effectively leads to up-regulation of mis-folded prion-like protein. The latter mode of amplification should properly be considered aberrantintracrine amplification. Finally, while the physiologic actions of normal intracrines are easily seen, those of pathological forms of intracrines are diverse and hard to discern. Thus, the physiology operative in Parkinson's disease and other prion-like neurodegenerative diseases might properly be considered an intracrine variant.

Practical implications of these ideas centers on the notion that amplification, if confirmed, represents a potential therapeutic target. The schema presented above suggests that lowering brain iron and zinc, through dietary manipulation, chelation, or even possibly phlebotomy, could be beneficial and should be evaluated in experimental models. A second therapeutic target would be the prevention of a physiologically increased IRPs/IRE binding caused by oxidative stress. If feasible, this could prevent increases in intracellular iron and secondarily prevent cell injury. Therefore, how oxidative stress, and in particular high iron conditions, up-regulate IRP activity is potentially an important area for study. Although generally assumed to increase cellular iron, in some cases nitric oxide does not. It is unclear what the result of inducing nitric oxide up-regulation in Parkinson's disease substania nigra neurons would be, but the possibility that it could produce a therapeutic dampening of IRP action seems worthy of exploration [84-89]. A third approach would be to lower intracellular zinc concentrations by, for example, targeting prion protein, a normal protein that transports zinc into cells. APP produces iron extrusion and the available data suggests that this extrusion function is impaired by zinc [51,54-57]. Prototype drugs exist for the reduction of cell-surface prion protein and it is possible that therapeutic agents based on these agents could have utility in Parkinson's disease as well as transmissible spongiform encephalopathies [13]. Prion protein transports both copper and zinc into cells and knocking it down could lower cellular zinc content. However, given reports that copper is reduced in Parkinson's disease substantia nigra, the reduction of surface prion protein, a copper transport protein, could further reduce cellular copper and prove to be a double edged sword. Deficient brain copper can lead to decreased ferroxidase activity and thereby increase tissue iron [90]. The role of copper metabolism in Parkinson's disease requires further study as do the respective roles of extracellular and intracellular copper and zinc. Inhibitors of specific zinc transporters potentially could, however, offer more specific therapeutic alternatives for reducing any detrimental effects of zinc in the substantia nigra [91].

Finally, two points dealing with disease initiation should be made. Alpha-synuclein increases with age in dopaminergic substantia nigra neurons in the absence of alpha-synuclein aggregation. This increase in alpha-synuclein is associated with decreased dopaminergic activity, perhaps related to the down-regulatory effects of alpha-synuclein on dopamine synthesis. Arguably, this tendency for alpha-synuclein to increase with age predisposes to alpha-synuclein mis-folding and to an increased probability of the development of Parkinson's disease. Therefore, the prevention of age-related alpha-synuclein up-regulation could lessen the likelihood of disease development. The enzyme kallikrein 6, also called neurosin, degrades alpha-synuclein in the brain and neurosin down-regulation is associated with increased alpha-synuclein content. If the age-related increase in alpha-synuclein reflects a decreased activity of neurosin, then stimulating the activity of this enzyme could be prophylactic, although it could be a double edged sword in that neurosin may enhance the production of pathological beta amyloid from amyloid precursor protein [92,93]. Because there is evidence that substantia nigra iron increases with age, age-related alpha-synuclein up-regulation could be iron-mediated [94]. Whether a feed-forward loop is involved in the absence of alpha-synuclein aggregation is unknown, but this possibility seems worthy of investigation. Also, the observation that in experimental models the pro-inflammatory bacterial endotoxin lipopolysaccharide can lead to neuronal alpha-synuclein accumulation suggests that lessening exposure to endotoxins may prove beneficial in reducing the development of disease [34]. Moreover, given the presence of bacterial lipopolysaccharides in the gut, the proposal that, in the development of Parkinson's disease, alpha-synuclein up-regulation, followed by aggregation, occurs first in the intestine with subsequent protein trafficking to the brain, is independent of, but consistent with, the intracrine mechanisms hypothesized here [34]. If abnormal alpha-synuclein can spread from the periphery to the central nervous system via afferent nerves, then the arguments presented here suggest that alpha-synuclein amplification occurs in those afferent nerves [34,95].

Conclusion

Here the basic characteristics of intracrine biology, on the one hand, and of the prion-like neurological disorders, on the other, have been reviewed and the similarities between them discussed. These similarities have implications for investigating the pathogenesis of Parkinson's disease. The potential role and importance of aberrantintracrine protein amplification in Parkinson's disease has been discussed. Indeed, because the mechanism of prion-like protein amplification in neurodegenerative disorders clearly differs from that of their normal homologues, and because the effect of intracrine action in those disorders is pathological rather than physiological, the biology operative in neurodegenerative disorders such as Parkinson's disease should properly be considered aberrant intracrine action. If confirmed, the intracrine-like nature of the prion-like neurodegenerative disorders could point to novel therapeutic interventions. At the same time, this review touches on the dual functionality of divalent metals in the pathogenesis of these disorders. Although it has long been assumed that the pro-aggregation actions of these metals facilitate pathology, here it is argued that in the case of Parkinson's disease iron and zinc participate in disease propagation by virtue of their effects on intracrine amplification. Similar arguments likely can be made in the case of other prion-like neurodegenerative disorders. If this suggestion is confirmed, additional therapeutic approaches would become available. That is, the recasting of Parkinson's disease and other neurodegenerative disorders as what might be called intracrine-like metallopathies could provide valuable leads for future progress.

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