Calcium Channel Antagonists as Disease Modifying Therapy for Parkinson's Disease: Therapeutic Rationale and Current Status

Tara Swart[1] and Michael J Hurley[2*]

[1]MIT Sloan Executive Education, Cambridge, MA 02142, USA

[2]Neuroepidemiology and Ageing Research, School of Public Health, Imperial College London, UK

*Corresponding author: m.hurley@imperial.ac.uk Michael Hurley PhD, Burlington Danes Building, Imperial College London, Hammersmith Campus, DuCane Road, London, W12 0NN, UK

Abstract

Parkinson's disease is a disabling hypokinetic neurological movement disorder with unknown aetiology in the majority of cases. Current pharmacological treatments, though effective at restoring movement, are only symptomatic and do nothing to slow the progression of the disease.

Electrophysiological, epidemiological and neuropathological studies have implicated $Ca_V1.3$ subtype calcium channels in the pathogenesis of the disorder and drugs (brain penetrant dihydropyridine calcium channel blockers) with some selectivity for this ion channel are neuroprotective in animal models of the disease.

Dihydropyridines have been safely used for decades to treat hypertension and other cardiovascular disorders. A phase II clinical trial found that isradipine was safely tolerated by Parkinson's disease patients and a phase III trial is currently underway to determine whether treatment with isradipine is neuroprotective and therefore able to slow the progression of Parkinson's disease.

This manuscript is a review of the current information about the use of dihydropyridines as therapy for Parkinson's disease, and discusses the possible mechanism of action of these drugs bringing to attention $Ca_V1.3$ calcium channels as a potential therapeutic target for neuroprotection in Parkinson's disease.

Key points

$Ca_V1.3$ calcium channels are implicated in the pathogenesis of Parkinson's disease.

Calcium channel blockers with selectivity for $Ca_V1.3$ calcium channels are neuroprotective in animal models of Parkinson's disease.

A phase III clinical trial is ongoing to test whether the calcium channel blocker isradipine is neuroprotective in Parkinson's disease

1. Introduction

Parkinson's disease is a progressive hypokinetic neurodegenerative neurological disorder characterised by bradykinesia, rigidity, akinesia, abnormal posture and resting tremor, together with non-motor functional deficits such as autonomic and sensorimotor dysfunction, cognitive decline, depression and sleep disturbances.[1] Increasing age is the greatest risk factor for Parkinson's disease – though approximately 10% of cases occur before age 50 – such that worldwide it affects up to 1% of people over 60 years of age, rising to above 2% for those aged over 80. In addition to the devastating effect Parkinson's disease has upon those afflicted with the disease and the people who care for them, increased life expectancy and the consequent ageing population, means Parkinson's disease is becoming an increasing burden on healthcare resources.[2] A treatment that can slow the inexorable process of the disease would therefore be a great benefit to both patients and society as a whole.

Current pharmacological treatments for Parkinson's disease are symptomatic and do nothing to slow or reverse the relentless progression of the disease (Table 1). Furthermore, they are associated with disabling motor and psychiatric side-effects which can limit their use or necessitate a reduction in dose to sub-optimal levels that do not adequately restore motor function.

The search for a disease-modifying pharmacological treatment (surgical and gene therapy approaches will not be discussed in this article) is hampered by not knowing the cause of the majority of cases of Parkinson's disease. Distinguishing Parkinson's disease from other forms of parkinsonism is difficult, particularly at early stages of the disease. In the past, patients presenting with some of the cardinal symptoms of Parkinson's disease and who had a good response to L-DOPA were generally given a diagnosis of Parkinson's disease. And although the definition and diagnostic criteria for Parkinson's disease diagnosis has been updated over the years to incorporate new findings[3], the diagnosis of Parkinson's disease can only be definitively confirmed post-mortem by neuropathological analysis showing loss of pigmented neurons in the substantia nigra, locus coeruleus and dorsal motor nucleus together with the presence of neuronal Lewy and pale bodies throughout the brain. Lewy bodies are abnormal aggregates of misfolded protein (predominantly alpha-synuclein, but they also contain other proteins such as ubiquitin, neurofilament proteins and tau) that form when the protein degradation system of the cell is dysfunction or overwhelmed, or when the alpha-synuclein is mutated to a form that favours formation of insoluble fibrils. The exact function of soluble (normal) alpha-synuclein is unknown, but it is thought to be involved in neurotransmitter release and mitochondrial function. Alpha-synuclein aggregates are also found in other diseases (synucleinopathies) such as dementia with Lewy bodies, multiple

system atrophy and progressive supranuclear palsy. Alpha-synuclein pathology can also be present in cases of Alzheimer's disease. Analysis of the neuropathological phenotype allows distinction of Parkinson's disease from other disorders which often present with identical symptoms (e.g. progressive supranuclear palsy and multiple system atrophy), but which have a different neuropathology (e.g. alpha-synuclein inclusions in oligodendrocytes). As such, Parkinson's disease is likely diagnosed for a range of disorders with overlapping symptoms the main one clearly being parkinsonism (bradykinesia, rigidity, akinesia, abnormal posture, resting tremor) with concomitant autonomic and sensorimotor dysfunction that often precedes motor symptoms.

The discovery that mutations (and later multiplications) of alpha-synuclein can cause monogenic forms of Parkinson's disease with Mendelian autosomal-dominant inheritance[4,5] enabled staining of Lewy bodies and neurites with antibodies raised against alpha-synuclein.[6] This led to neuropathological Braak alpha-synuclein staging of the disease and the hypothesis of spread of alpha-synuclein pathology through brain[7] and ultimately to the recognition of a new group of neurodegenerative diseases, the synucleinopathies.

For familial forms of the disease - 21 genes or genetic loci are found to be causative of, or susceptibility factors for, Parkinson's disease[8] - cases are often early-onset and have a different presentation (e.g. prominent dystonia) and progression of symptoms compared to idiopathic forms of the disorder. But, in most instances, the contribution of mutations to Parkinson's disease is far from clear due to limited gene penetrance and the wide range of phenotypes of the disease. Overall, Parkinson's disease caused by known monogenic mutations represents approximately 30% of familial and 3-5% of sporadic cases of the disorder.[9]

A central theme for the cause of Parkinson's disease, discovered in the late 1980's and before a genetic basis for Parkinson's disease was considered likely, was oxidative stress leading to death of catecholamine neurons in the midbrain.[10] And, when known, the function of genes that cause or increase risk for Parkinson's disease affect protein turnover or metabolism[11]. The former could cause metabolic stress due to congestion of processes within the cell leading to inefficient function, while the latter presumably directly induce oxidative stress. Oxidative stress likely originates from many different sources such as increased free radicle production in mitochondria due to defects in enzymes of the respiratory chain, mutations in proteins that regulate mitochondrial function (e.g. parkin, PINK1, DJ-1), decreased expression of antioxidants (e.g. glutathione), abnormal iron accumulation, reduced trophic support (e.g. decreased GDNF (glial cell derived neurotrophic

factor) or inappropriate inflammatory cytokines released from activated microglia (neuroinflammation). Anti-oxidants, anti-inflammatory drugs, iron chelating agents and intracerebral GDNF delivery have been used to directly mitigate these processes and restore motor function effectively in animal models of Parkinson's disease, but with less success when used as treatments, or in clinical trials, for patients with Parkinson's disease. The physiological use of calcium to maintain autonomous pacemaking activity in neurons vulnerable to degeneration also contributes to mitochondrial oxidant stress, as does the distribution of calcium binding proteins (e.g. calbindin) which have an inverse correlation between their expression and the risk of degeneration of a neuron in Parkinson's disease. As such, altered calcium homeostasis is also now believed to be a major source of oxidative stress and will be discussed further below. Abnormal protein accumulation also stems from many different sources such as misfolded or mutated (e.g. alpha-synuclein) proteins and defects in proteasome and lysosome function or the ubiquitination pathway. Abnormal protein accumulation might be effectively treated with antibodies against the protein, as has been tried to reduce amyloid plaques in Alzheimer's disease, or by enhancing the action of lysosome mediated autophagy.[11]

Both types of dysfunction lead to cell death in vulnerable neurons and it is likely that patients with Parkinson's disease have a combination of factors which cause oxidative stress and abnormal protein accumulation in the aetiology of their disease. But what makes some neurons vulnerable compared to other neurons or types of cell in brain? The brain is approximately 50% neurons, with the remainder comprised of glia (astrocyte, microglia, oligodendrocytes) and blood vessels etc.[12] Neurons are post-mitotic and do not regenerate (with the possible exception of some cells in the hippocampus) and therefore they have to last a lifetime. Hence any homeostatic problems they have will be cumulative over the lifetime of the individual. Furthermore, the specialization of neurons, i.e. neurotransmission, makes them particularly vulnerable compared to other cell types. This is particularly so for projection neurons which link different brain regions, as opposed to short interneurons, because they have long fine axons with large dendritic and synaptic fields and are often poorly myelinated.[13] So, unless manufacture and degradation occurs locally in the synapse, cellular components have a long way to travel between the cell body and the axon terminal. Which itself is an energy demanding process that would place metabolic stress upon the neuron.

As stated above, both general mechanisms (protein turnover and oxidative stress) are likely to play a role in cell death in Parkinson's disease. Cases of Parkinson's disease that result predominantly from excessive mitochondrial oxidant stress are more likely to respond to calcium channel blocker therapy while others, where this mechanism of toxicity is less

dominant, may show less clinical benefit. But, there is no means of routinely determining the extent of alpha-synuclein pathology in living patients because alpha-synuclein imaging techniques are still experimental and similarly direct detection of oxidative stress in living brain is not possible. As such, it is not currently possible to stratify patients with Parkinson's disease. However, because calcium channel blocking drugs are available and approved for use in humans and have a good safety record, using them therapeutically to modulate intracellular calcium levels pharmacologically is currently more easily achievable than trying to reduce alpha-synuclein accumulation using, for example, experimental monoclonal antibodies against the (presumably) aggregated form of the protein and therefore use of calcium channel blocking drugs to treat Parkinson's disease is the subject of this review.

2 The role of Ca_V1 channels in Parkinson's disease

Calcium plays a central role in the normal functioning of neurons and is also involved in the many cellular processes (e.g. oxidative stress, mitochondrial impairment, proteasomal dysfunction, excitotoxicity, neuroinflammation, apoptosis) that can lead to cell death in Parkinson's disease.[14,15,16,17,18,19] Ca_V1 calcium channels are distinguished from other calcium channels by their long (L) lasting inward currents during depolarization (they were previously called L-type calcium channels) and their high sensitivity to dihydropyridines (L-type calcium channel blockers), which have been used for many years to treat hypertension and other cardiovascular diseases. But, use of calcium by a cell comes at a cost, because high concentrations of intracellular calcium are toxic and extrusion of calcium from the cell and sequestration of calcium into intracellular stores requires energy that is derived from mitochondrial oxidative phosphorylation. The electrochemical gradient for calcium across cellular membranes is much larger (1000×) than that of monovalent ions, so any process that uses calcium in the cell is very energy demanding. So, another feature of neurons vulnerable to neurodegeneration, in addition to their morphology, is that they have high energy requirements, which requires efficient mitochondrial function together with a good calcium buffering capacity to prevent damage through oxidative stress or excitotoxicity.[20] One means of calcium buffering is achieved by calcium binding proteins and the observation that degenerating neurons in the substantia nigra were mainly in areas with low levels of the calcium binding protein calbindin-D28k, suggested a role for calcium in neuronal susceptibility.[21,22,23] However, in addition to the varied distribution of calcium buffering found in brain, recent studies have further implicated Ca_V1 in the pathogenesis of Parkinson's disease.

Another shared feature of midbrain dopamine neurons and the other brainstem nuclei that degenerate in Parkinson's disease is that they are autonomously active, with prominent

transmembrane calcium currents that generate regular, slow, broad action potentials (2–4 Hz) in the absence of synaptic input.[20] This so called pacemaking activity maintains basal neurotransmitter levels in regions that are innervated by these neurons, i.e. dopaminergic tone. While most neurons rely exclusively on monovalent cation channels to drive such pacemaking, studies in animals indicate that neurons vulnerable to neurodegeneration in the substantia nigra pars compacta and dorsal motor nucleus of the vagal nerve preferentially use $Ca_V1.3$ for pacemaking. Whereas, dopaminergic neurons in the ventral tegmental area, that is adjacent to the substantia nigra, that are less affected by neurodegeneration in Parkinson's disease rely instead upon sodium ion entry for maintenance of conductance oscillations. Furthermore, other neurons that use $Ca_V1.3$ channels that do not degenerate in Parkinson's disease, e.g. striatal spiny neurons, do not exhibit pacemaking but are instead only episodically activated.[24,25,26,27,28] The use of calcium rather than sodium ions for pacemaking requires much more energy expenditure in order to maintain a safe non-toxic intracellular calcium concentration. And, combined with the structural phenotype of susceptible neurons and differential calcium binding protein distribution described above, these factors may be the reason why some neurons degenerate more in affected areas of brain (e.g. ventral–lateral zone > dorsal tier of the substantia nigra) in Parkinson's disease while other regions containing similar neuron types are less affected (e.g. ventral tegmental area). In Parkinson's disease, where mitochondrial dysfunction is evident, the reliance on $Ca_V1.3$ channels, should it also occur in humans, together with the other phenotype characteristics mentioned above may make the substantia nigra pars compacta neurons and other brainstem projection nuclei more susceptible to calcium mediated excitotoxicity.[10,20,29] The reliance on $Ca_V1.3$ channels for pacemaking in the substantia nigra may also explain the decline in dopaminergic neurons in this region with advancing age[30] but doesn't explain why we don't all get Parkinson's disease. However, Parkinson's disease is not just accelerated ageing because the pattern of cell loss is different in post-mortem brain from non-parkinsonian elderly people to that seen in brain from patients dying with Parkinson's disease[31]. And as stated above, a number of mechanisms (oxidative stress, mitochondrial impairment, proteasomal dysfunction, excitotoxicity, neuroinflammation, apoptosis) are involved in the neurodegeneration that occurs in Parkinson's disease. However, it should be noted that $Ca_V1.3$ channels are dispensable in maintaining pacemaking activity because when dendritic calcium oscillations are eliminated by blockade with dihydropyridines, pacemaking still occurs through a combination of voltage-dependent sodium, potassium and HCN (hyperpolarization and cyclic nucleotide-activated cation) channels, which are all also expressed by autonomously active neurons in the midbrain and brainstem.[32,33,34] Consequently, blocking $Ca_V1.3$ channels should provide a reduction in oxidative stress in

surviving neurons (and hence provide neuroprotection) but should not affect their autonomous activity and physiological function (Fig. 1).

In addition to the unusual electrophysiology of midbrain catecholamine neurons another separate line of evidence implicates Ca_V1 in the pathogenesis of Parkinson's disease. That is, retrospective analysis of patients treated with Ca_V1 channel blocking drugs for hypertension and cardiac arrhythmias has found a decreased risk for Parkinson's disease in patients treated with dihydropyridines that cross the blood brain barrier, which suggested a neuroprotective effect of such drugs and implied a pathogenic role for the Ca_V1 channel subtype in Parkinson's disease.[35,36]

3 Localization of Ca_V1 in brain and Parkinson's disease

$Ca_V1.2$ and $Ca_V1.3$ are the predominant subtype of Ca_V1 found in central nervous system and they have a widespread distribution throughout the brain (Table 2). Whereas $Ca_V1.1$ and $Ca_V1.4$ are only expressed in skeletal muscle and the retina respectively. Although there are reports of very low levels (detected by polymerase chain reaction) of transcripts for $Ca_V1.1$ and $Ca_V1.4$ being present in brain, their functional significance is uncertain and the detection of them may be due to the high sensitivity of PCR and the presence of other cell types (e.g. lymphocytes) in non-perfused brain preparations.[17,49,50,56,57]

Early studies that examined the expression of Ca_V1 channels in post-mortem brain from patients who died with Parkinson disease used radiolabelled dihydropyridine ligands. Consequently, the studies did not differentiate between isoforms of Ca_V1 channel. No change in the density of [^{3}H]PN 200–110 (isradipine) binding sites in striatum from patients dying with Parkinson's disease was found by Watson and colleagues[58]. Likewise, Sen and co-workers[59] found that the B_{max} (total number of binding sites) and K_d (affinity) for [^{3}H]PN 200–110 binding sites was not significantly altered in striatum, thalamus, temporal cortex or occipital cortex of parkinsonian brain. Both studies also examined striatal tissue from patients dying from Huntington's disease and measured a significant reduction in Ca_V1 channel binding sites in this region. These data indicate that Ca_V1 channels were predominantly located on neurons intrinsic to the striatum, since striatal medium-sized spiny neurons degenerate in Huntington's disease.[60] However, an earlier study by Nishino and colleagues[61] using [^{3}H]nitrendipine reported a decrease in dihydropyridine binding sites in striatum and substantia nigra from patients dying with Parkinson's disease, which suggested that Ca_V1 channels were present on neurons projecting to the striatum from the substantia nigra. The reason for the differing results is unknown, but probably reflects differences in the binding characteristics of the radioligands used to label the Ca_V1 channel isoforms in brain.

Since Nishino and co-workers[61] measured no change in prefrontal cortex or globus pallidus, it seems unlikely that the post-mortem delay or agonal state of the Parkinson's disease patients prior to death was the cause of the reduction in [^{3}H]nitrendipine binding sites.[17]

More recently expression of Ca_V1 subtypes was examined in post-mortem human brain by immunohistochemistry and *in situ* hybridization in areas vulnerable and resistant to neurodegeneration in Parkinson's disease. The expression of $Ca_V1.3$ was found to be elevated compared to age-matched controls throughout the brain. Such that the ratio of Ca_V1 subtypes ($Ca_V1.2$:$Ca_V1.3$) differed throughout the brain in patients with Parkinson's disease compared with control subjects, in favour of an increased use of $Ca_V1.3$. Increased use of $Ca_V1.3$ would add to the metabolic burden for cells that rely on this Ca_V1 subtype for electrical activity and could therefore render specific neuronal populations more vulnerable to neurodegeneration.[42,43] Furthermore, the increased expression of $Ca_V1.3$ in cerebral cortex of early stage Parkinson's disease cases (Braak alpha-synuclein stage 3 and 4), before the appearance of pathological changes in these regions, supports the view that disturbed calcium homeostasis is an early feature of Parkinson's disease and not just a compensatory consequence to the neurodegenerative process.[42,43]

4 Calcium channel blockers and Parkinson's Disease risk

A number of studies have examined whether chronic treatment with calcium channel blockers for hypertension or cardiovascular disorders might reduce the risk of developing Parkinson's disease by acting as neuroprotective agents, often with conflicting outcomes. Thus, some studies found that calcium channel blocking drugs reduced the risk of Parkinson's disease,[35,36,62,63] whereas others did not find a significant effect.[64,65,66,67] However, the consensus based upon systematic review of all available literature is that treatment with calcium channel blockers does appear to reduce the risk of Parkinson's disease[68,69] although the mechanism by which they do so is not completely understood.

Ca_V1 subtypes, in addition to regulation of neuronal electrical activity and modulation of gating of other ion channels, control numerous cellular processes such as neurotransmitter release, neuronal survival, kinase activation, neurite outgrowth and gene transcription by either influx of calcium into cells following membrane depolarization or release from intracellular stores and are found on non-neuronal cells in brain which are not electrically active.[17,70,71] As such, although contentious, it is worth considering additional (or alternative) mechanisms to the prevention of calcium influx into spontaneously active cells, whereby Ca_V1 blockade could lead to neuroprotection. Is neuroprotection observed in animal models of Parkinson's disease following treatment with isradipine achieved purely through a

reduction in mitochondrial oxidant stress,[25] or is there also an anti-inflammatory component mediated through modulation of glial cell activity.[72,73,74] and hence neuroinflammation, through actions on Ca_V1 subtypes present on non-neuronal cells? Also, in addition to a direct effect on neuroinflammation through inhibition of glial cell activity via blockade of Ca_V1 subtypes, reducing blood pressure per se may also be neuroprotective. Particularly in cases of Parkinson's disease where there is co-existent vascular pathology. Hypertension may lead to oxidative stress and inflammation in brain through actions on the central renin-angiotensin system.[75] And an association between hypertension and Parkinson's disease risk was described in women[76], so there could be an additional indirect mechanism by which calcium channel blockers are neuroprotective other than by decreasing intracellular calcium directly. More selective $Ca_V1.3$ blocking ligands have recently been described which should provide a more potent means of reducing mitochondrial oxidant stress in rhythmically firing catecholamine neurons of the midbrain and brain stem[77]. Studies using such ligands in animal models of Parkinson's disease and ultimately in human trials should resolve this issue.

5 Calcium channel blocker clinical trials

Preclinical studies in rodent and non-human primate models of Parkinson's disease demonstrated that pre-treatment with isradipine (which has an almost equimolar affinity for $Ca_V1.2$ and $Ca_V1.3$) or nimodipine could protect dopamine neurons from MPTP- and 6-OHDA-induced toxicity. And it was concluded that this occurred through an action on nigral $Ca_V1.3$ channels that resulted in less calcium entering the cells with a consequent reduction in the energy used and hence oxidant stress required to control intracellular calcium levels.[25,78,79,80] Since isradipine was already FDA-approved for use in hypertension, a Phase II safety and tolerability trial was rapidly organized to determine the safest and highest that could be used in Parkinson's disease patients.[81] The study found that isradipine tolerability was dose dependent with dizziness and peripheral oedema being the most common side-effect. However, there was no significant symptomatic benefit observed versus placebo. But, since the study participants were early cases of Parkinson's disease that did not require dopaminergic medication, the measured effect did not exclude the possibility of clinical benefit if isradipine was given as a long-term adjunct to traditional (dopaminergic) therapies in patients with more advanced disease.[81] As such, a randomised multi-centre double-blind placebo-controlled Phase III clinical efficacy trial (STEADY-PD III, ClinicalTrials.gov identifier NCT02168842) to test the ability of isradipine to slow progression of Parkinson's disease is currently underway, with results expected in early 2019.

6. Conclusions

The above has provided an overview of why disturbed calcium homeostasis could have a role in the pathogenesis of Parkinson's disease and how blockade of the $Ca_V1.3$ subtype could be a means of mitigating neurodegeneration. The variable disease phenotype could confound the outcome of the isradipine clinical trial. Particularly since the trial recruited early cases and these patients might turn out to have an atypical or familial form of the disorder. This means that even if the trial fails, which is so often the case for putative Parkinson's disease treatments which work well in pre-clinical models, the pursuit of calcium channel antagonists should not be abandoned.

Compliance and ethical standards

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Conflicts of interest

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Table 1 Drugs used to treat Parkinson's disease

Drug or class of drug	**Principal mechanism of action**	**Use**
L-DOPA/carbidopa	increases brain dopamine levels	Restore motor function
Dopamine receptor agonists	stimulate central dopamine receptors	Restore motor function
Muscarinic cholinergic receptor antagonists	block central muscarinic acetylcholine receptors	Reduce tremor, rigidity and drooling
Amantadine	weak NMDA receptor antagonist	Dyskinesia and tremor
Monoamine oxidase B inhibitor	Decrease monoamine degradation	Adjunct to L-DOPA in early Parkinson's
Catechol-O-methyl transferase inhibitor	Decrease catecholamine degradation	Adjunct to L-DOPA

Abbreviations: L-DOPA, L-3,4-dihydroxyphenylalanine; NMDA, N-methyl-D-aspartate

Table 2

Regional distribution of Ca_V1 voltage-gated calcium channels in brain (mRNA/protein)*.

	$Ca_V1.1$	$Ca_V1.2$	$Ca_V1.3$	$Ca_V1.4$
Olfactory bulb	?	++/+	++/+	?
Cerebral cortex	(+)/?	++/++	++/++	(+)/?
Striatum	(+)/?	+/+	+/+	(+)/?
Nucleus basalis	?	?/++	?/++	?
Amygdala	?	+/+	+/+	?
Hippocampus	(+)/?	++/++	++/++	(+)/?
Thalamus	?	+/+	−/?	?
Habenulae	?	−/?	+/?	?
Hypothalamus	?	−/?	+/?	?
Substantia nigra	(+)/?	++/++	++/++	?
Locus coeruleus	?	++/++	++/++	?
Dorsal motor nucleus	?	?/++	?/++	?
Superior colliculus	?	−/?	++/+	?
Inferior colliculus	?	−/?	−/?	?
Cerebellum	(+)/?	++/++	++/++	(+)/?

− = absent, + = low, ++ = moderate, +++ = high, ( ) = questionable, ? = unknown.

*Data from references: 34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52
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Abbreviations: Ca_V1, L-type voltage-gated calcium channel subtype 1; mRNA, messenger ribonucleic acid

Figure 1

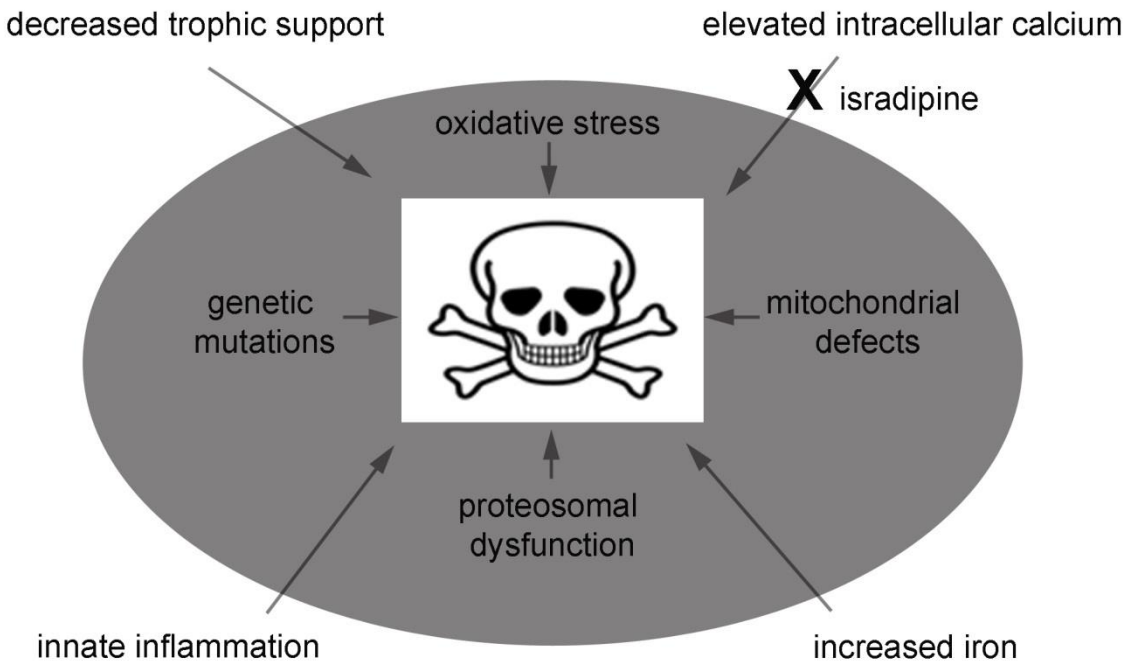

Figure 1 Legend

Numerous molecular mechanisms contribute to the neurodegenerative process in Parkinson's disease and their damage is additive. Preventing excessive calcium influx by blockade of $Ca_V1.3$ channels with dihydropyridine drugs (e.g. isradipine) would reduce metabolic stress caused by the need to reduce calcium concentrations to non-toxic levels and may therefore result in neuroprotection. The pacemaking function of surviving neurons would not be affected because other ion channels are also able to perform this function.