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Connecting Ca²⁺ and lysosomes to Parkinson disease

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Abstract

The neurodegenerative movement disorder Parkinson disease (PD) is prevalent in the aged population. However, the underlying mechanisms that trigger disease are unclear. Increasing work implicates both impaired Ca²⁺ signalling and lysosomal dysfunction in neuronal demise. Here I aim to connect these distinct processes by exploring the evidence that lysosomal Ca²⁺ signalling is disrupted in PD. In particular, I highlight defects in lysosomal Ca²⁺ content and signalling through NAADP-regulated two-pore channels in patient fibroblasts harbouring mutations in the PD-linked genes, *GBA1* and *LRRK2*. As an emerging contributor to PD pathogenesis, the lysosomal Ca²⁺ signalling apparatus could represent a novel therapeutic target.

Keywords

Ca²⁺; Lysosomes; NAADP; TPC1; TPC2; TRPML1; Parkinson disease; *LRRK2*; *GBA1*

1 Introduction

Parkinson disease (PD) is a common, disabling and incurable neurodegenerative movement disorder. Over 1% of individuals aged 65 or over develop PD and prevalence increases to 4% by the age of 85 (de Lau & Breteler, 2006). The progressive decline in the control of voluntary movement, which is the principle symptom of the disease, is caused by the selective death of dopaminergic neurons in the substantia nigra pars compacta (SNc). Pathologically, these neurons develop intraneuronal inclusions, composed of alpha-synuclein (α -syn), called Lewy bodies (Braak *et al*, 2003).

As well as advanced age, several other factors have been linked to PD onset. Notably, genetic mutations account for 10-15% of PD cases (reviewed in Mullin & Schapira, 2015). To date, 11 genes have been clearly linked with PD (Brás *et al*, 2015) and a number of other susceptibility loci have been identified through genome-wide association studies (such as Nalls *et al*, 2014). However, the cause of the disease cannot be determined for the majority of patients.

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Conflict of interest

The author declares no conflict of interest.

Numerous cellular and biochemical dysfunctions are involved in PD pathogenesis (recently reviewed in Michel *et al*, 2016). Increasing evidence identifies both deregulated Ca^{2+} signalling and lysosomal dysfunction as central contributors to neuronal death. Recent studies have connected these pathologies by establishing that Ca^{2+} signalling from lysosomes is disrupted in PD. Here, I examine this evidence and discuss how impaired lysosomal Ca^{2+} signalling might contribute to PD.

2 Lysosomes as Ca^{2+} stores

Ca^{2+} is a ubiquitous, abundant and highly versatile signalling cation. A vast range of physiological events, from the beginning of life to cell death, are co-ordinated by changes in cytosolic Ca^{2+} . In neurons, Ca^{2+} regulates numerous functions, such as synaptic release (Brini *et al*, 2014). At rest the cytosolic Ca^{2+} concentration of a cell is low (typically 100 nM), but upon stimulation Ca^{2+} levels can rise 10-100 fold (Berridge *et al*, 2000). These Ca^{2+} signals can originate from both the extracellular environment and intracellular Ca^{2+} stores.

The endoplasmic reticulum (ER) is a well-characterised Ca^{2+} store (reviewed in Stutzmann & Mattson, 2011). Increasing work now identifies acidic organelles, like lysosomes, endosomes and secretory vesicles, as Ca^{2+} stores too (Figure 1). Much of this evidence was based on studies using the lysosomal-permeabilising agent GPN (glycyl-L-phenylalanine 2-naphthylamide, Haller *et al*, 1996) which evokes Ca^{2+} signals in a variety of cell types, including neurons (Pandey *et al*, 2009; Dickinson *et al*, 2010; Coen *et al*, 2012). Ca^{2+} indicators trafficked to the lysosomal lumen following endocytosis provide more direct evidence that lysosomes are Ca^{2+} stores. Such indicators report a luminal Ca^{2+} concentration similar to the ER, approximately 500 μM (Christensen *et al*, 2002; Lloyd-Evans *et al*, 2008).

Lysosomes are the target of the potent Ca^{2+} mobilising messenger NAADP (Nicotinic acid adenine dinucleotide phosphate, Churchill *et al*, 2002). Despite recent controversies (Wang *et al*, 2012; reviewed in Marchant & Patel, 2013), it is largely accepted that NAADP releases lysosomal Ca^{2+} through Two-pore Channels (TPC1 and TPC2, Calcraft *et al*, 2009; Brailoiu *et al*, 2009a; Zong *et al*, 2009; reviewed in Patel, 2015). These Ca^{2+} , Na^{+} and H^{+} permeable channels are members of the voltage-gated ion channel superfamily (Rahman *et al*, 2014) that are additionally gated by $\text{PI}(3,5)\text{P}_2$ (Phosphatidylinositol 3,5-bisphosphate, Wang *et al*, 2012), a phosphoinositide involved in membrane trafficking. Recent evidence has implicated TPCs in the pathogenesis of numerous diseases, such as Ebola infection (Sakurai *et al*, 2015), ischemia reperfusion injury (Davidson *et al*, 2015), liver disease (Grimm *et al*, 2014), cancer (Favia *et al*, 2016; Nguyen *et al*, 2017) and PD (see below).

Other Ca^{2+} -permeable channels reside on lysosomes (reviewed in Xiong & Zhu, 2016). These include members of the transient receptor potential (TRP) ion channel family – TRP mucolipins (TRPML1, TRPML2 and TRPML3) and TRPM2 (Lange *et al*, 2009; Sumoza-Toledo *et al*, 2011) as well as ATP-gated ionotropic receptors (P2X4, Qureshi *et al*, 2007; Huang *et al*, 2014) and voltage gated Ca^{2+} channels (VGCCs; Tian *et al*, 2015). TRPML1 is of particular interest as mutations in its corresponding gene (*MCOLN1*) are responsible for the autosomal recessive lysosomal storage disorder (LSD) Mucopolidosis IV (MLIV, Bargal

et al. 2000). MLIV is characterised by severe neurodegeneration and associated with disrupted endo-lysosomal trafficking, substrate degradation and lysosomal biogenesis (reviewed in Venkatachalam *et al.* 2015). Like TPCs, TRPMLs are permeant to several cations and gated by PI(3,5)P₂ (Dong *et al.* 2010) and possibly NAADP (Zhang & Li, 2007; but see Yamaguchi *et al.* 2011).

As well as mediating Ca²⁺ release, lysosomes have an emerging role in tempering cytosolic Ca²⁺ signals. Recently, lysosomes have been shown to rapidly sequester Ca²⁺ released from the ER (López-Sanjurjo *et al.* 2013) which could be important for their re-filling (Garrity *et al.* 2016). However, the precise mechanism behind lysosomal Ca²⁺ uptake remains elusive. Since both ATP (Klemper, 1985) and a proton gradient are likely to be important (Christensen *et al.* 2002) Ca²⁺-ATPases and Ca²⁺/H⁺ exchangers (CAXs) have been proposed (reviewed in Patel & Docampo, 2010). Although CAXs have been recently described in many vertebrates (Melchionda *et al.* 2016), no human homologue has yet been identified.

In summary, lysosomes store Ca²⁺ and release this signalling cation via multiple channel mechanisms. Emerging evidence identifies that deregulated signalling through these channels contributes to a range of diseases.

3 Functions of lysosomal Ca²⁺ signalling

The connection between lysosomal ion channels and disease underscores the physiological importance of Ca²⁺ signalling from these organelles. Indeed, lysosomal Ca²⁺ signals regulate various cellular functions, including trafficking (discussed below), autophagy (Pereira *et al.* 2011), and neuronal differentiation (Brailoiu *et al.* 2006) and depolarisation (Brailoiu *et al.* 2009b). The Ca²⁺ signals generated by lysosomes can be confined to a small (10-100 nm) local area or propagate globally throughout the entire cell (Bootman *et al.* 2001).

3.1 Local lysosomal Ca²⁺ signalling

The endo-lysosomal system is dynamic and the fusion of endocytic compartments is necessary for trafficking and appropriate organelle function. It has been long-accepted that the fusion (and fission) of endo-lysosomes requires localised lysosomal Ca²⁺ release (Pryor *et al.* 2000). In this study fast, but not slow, acting Ca²⁺ chelators inhibited endo-lysosomal fusion events. These results suggest that the Ca²⁺ signal must be close to the sensor (i.e. localised) because it can escape buffering by the slower chelator. Much research has focused on TRPMLs as the channels responsible for these local Ca²⁺ signals (reviewed in Li *et al.* 2013). This attention is justified as trafficking deficiencies are a prominent in MLIV. Importantly, enhancing TRPML activity with recently developed agonists (Shen *et al.* 2012) has corrected trafficking defects in not only MLIV (Chen *et al.* 2014), but also HIV-associated dementia (Bae *et al.* 2014) and another LSD, Neimann-Pick disease type C (NPC, Shen *et al.* 2012).

Ca²⁺ release from TPCs has also been implicated in endocytic trafficking (reviewed in Marchant & Patel, 2015). For instance, TPCs regulate the transit of cholesterol (Grimm *et al.*

2014) and the trafficking of internalised growth factor receptors (Ruas *et al*, 2014). Furthermore, retrograde trafficking from endosomes to the Golgi complex (Ruas *et al*, 2010) might also require TPCs. In *Xenopus* oocytes, TPC2 was shown to regulate lysosomal trafficking through interactions with Rab7; an established trafficking GTPase (Lin-Moshier *et al*, 2014). Many of these TPC-related trafficking events are known to involve local Ca^{2+} signalling, because only fast acting Ca^{2+} chelators mimic trafficking phenotypes (Grimm *et al*, 2014; Lin-Moshier *et al*, 2014). Recent proteomic screens revealed that TPCs additionally interact with syntaxins (Lin-Moshier *et al*, 2014; Grimm *et al*, 2014) – members of the SNARE protein family which is required for membrane fusion.

In addition to localised Ca^{2+} signals, phosphoinositides (such as $\text{PI}(3,5)\text{P}_2$) also regulate endo-lysosomal membrane traffic (Mayinger, 2012). Since both TRPMLs and TPCs are regulated by $\text{PI}(3,5)\text{P}_2$, these channels likely integrate Ca^{2+} and phosphoinositide signals to control vesicle trafficking. As points of convergence for trafficking cues, lysosomal Ca^{2+} channels could be considered “trafficking hubs”.

3.2 Global lysosomal Ca^{2+} signalling

Due to their relatively small size, lysosomes generate localised Ca^{2+} signals. Nevertheless, these acidic organelles still contribute to global Ca^{2+} signalling through communication with the ER. According to the “trigger hypothesis”, local release of Ca^{2+} from the lysosomes sensitises ER Ca^{2+} release channels and consequently the ER, as the more substantial store, amplifies the initial lysosomal signal (reviewed in Penny *et al*, 2015). Numerous studies have reported that NAADP uses this form of coupling (Churchill & Galione, 2000; Brailoiu *et al*, 2005, 2009b; Kinnear *et al*, 2004; Cancela *et al*, 1999) and recently we have shown that TRPML channels are also capable of evoking secondary Ca^{2+} release from the ER (Kilpatrick *et al*, 2016b). The localised nature of the lysosomal Ca^{2+} signal suggests that the ER and lysosomal Ca^{2+} apparatus must be closely associated to facilitate coupling. Our identification of membrane contact sites (apposing membranes separated by <30 nm) between lysosomes and the ER is of relevance here (Kilpatrick *et al*, 2013).

Drawing these studies together, local and global lysosomal Ca^{2+} signals regulate a variety of cellular functions. It therefore follows that these functions might be disrupted in diseases where lysosomal Ca^{2+} signalling is deregulated.

4 Ca^{2+} and lysosomal dysfunction in Parkinson disease

The precise mechanism(s) that triggers PD is unclear. An array of biochemical and cellular dysfunctions are known to contribute to this disease, including oxidative, mitochondrial and ER stress (reviewed in Michel *et al*, 2016). Recent evidence highlights Ca^{2+} signalling and lysosome dysfunction as central mediators of PD pathology.

4.1 Ca^{2+} signalling in Parkinson disease

As mentioned above, loss of SNc neurons is the core pathology of PD. However, why these neurons, and not others, succumb has been a matter of debate. One potential mechanism involves Ca^{2+} . Unlike most other neurons in the brain, SNc neurons express an uncommon L-type voltage gated Ca^{2+} channel (VGCC) isoform, $\text{Ca}_v1.3$ (Chan *et al*, 2007). These

channels possess a distinct subunit, which opens during hyperpolarisation (Xu & Lipscombe, 2001). Since SNc neurons are autonomously active (Grace & Bunney, 1983), $\text{Ca}_v1.3$ subjects these neurons to an excessive influx of Ca^{2+} (Guzman *et al*, 2009). This Ca^{2+} exposure comes at an energetic cost to mitochondria as ATP is needed to drive Ca^{2+} out of the cytosol against its concentration gradient. As a result, neurons experience oxidative stress (Guzman *et al*, 2010) which might make SNc neurons less tolerant to stressors.

Other research has identified intracellular Ca^{2+} defects in PD. For instance, studies have shown a reduced expression of Ca^{2+} buffering proteins (such as Calbindin-D28K) in SNc neurons (Yamada *et al*, 1990) and compromised mitochondrial Ca^{2+} homeostasis in PD models (recently reviewed in Cieri *et al*, 2016). Altered mitochondrial Ca^{2+} dynamics have been linked to the impaired formation of membrane contact sites between the mitochondria and ER. Overexpression of parkin (Calì *et al*, 2013), DJ-1 (Ottolini *et al*, 2013) and α -syn (Calì *et al*, 2012) (each encoded by genes associated with PD) increase the number of contacts, which favours the transfer of ER Ca^{2+} to the mitochondria. Appropriate ER-mitochondrial communication is required to maintain mitochondrial bio-energetics, Ca^{2+} homeostasis and cell survival (reviewed in Paillusson *et al*, 2016). As the so-called “last straw” a disruption to these contacts, or indeed any other component of the Ca^{2+} signalling toolkit, could cause neurodegeneration in SNc neurons which are already vulnerable due to excessive Ca^{2+} influx.

In summary, defects in Ca^{2+} influx and intracellular homeostasis might contribute to PD and therefore agents modifying Ca^{2+} levels could represent viable therapeutic targets. Indeed, epidemiological studies have identified that L-type VGCC antagonists (such as dihydropyridines), which treat hypertension, reduce the risk of developing PD (Becker *et al*, 2008; Ritz *et al*, 2010; Pasternak *et al*, 2012; Lang *et al*, 2015). Therefore, these antagonists, some of which can cross the blood brain barrier (Ritz *et al*, 2010), could be repurposed as a PD treatment. A large scale NIH clinical trial addressing this hypothesis is currently ongoing, although we should consider alternative options as preliminary results have not been encouraging (Marras *et al*, 2012; Parkinson-Study-Group, 2013).

4.2 Lysosomes in Parkinson disease

Aside from their emerging role in Ca^{2+} signalling, lysosomes are at the centre of endocytic, autophagic and secretory pathways. With such an influential position it is perhaps unsurprising that lysosomal dysfunction has been reported in numerous diseases (reviewed in Appelqvist *et al*, 2013). Since lysosomes degrade α -syn through chaperone-mediated autophagy (Cuervo *et al*, 2004), merely the accumulation of α -syn (a defining feature of PD) implicates lysosomal dysfunction in PD. Many have identified other lysosomal deficiencies in PD (recently reviewed in Bourdenx & Dehay, 2016). In particular, studies have shown robust reductions in lysosomal markers within the SNc neurons of sporadic PD patients (Chu *et al*, 2009) and a toxin-induced (MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) murine model of PD (Dehay *et al*, 2010).

Some of the most convincing evidence linking lysosomes to PD is genetic. Namely, mutations in *GBA1*, a gene that encodes the lysosomal enzyme glucocerebrosidase which

underlie the recessive LSD Gaucher disease (GD), have been identified as the most common genetic risk factor for PD (Sidransky *et al*, 2009). Mutations disrupt the folding and trafficking of this lysosomal enzyme which causes a build-up of substrate (glucocerebroside) in the lysosomes (Ron & Horowitz, 2005). It has been suggested that by harming lysosomes, mutations in *GBA1* disrupt autophagy and allow toxic substances, like damaged mitochondria (Osellame *et al*, 2013) and α -syn (Mazzulli *et al*, 2011), to accumulate. In SNc cells which are not very tolerant to stress, such accumulation could initiate neurodegeneration.

Autosomal dominant mutations in *LRRK2* are another common genetic cause of PD (Paisán-Ruiz *et al*, 2004; Zimprich *et al*, 2004; Di Fonzo *et al*, 2006). *LRRK2* encodes leucine-rich repeat kinase 2 (LRRK2) a multidomain enzyme with kinase and GTPase activity. Although the exact function(s) of LRRK2 is unknown, it has been shown to associate with membranes of the endo-lysosomal system (Biskup *et al*, 2006; Alegre-Abarrategui *et al*, 2009; Higashi *et al*, 2009; Papkovskaia *et al*, 2012). LRRK2 also interacts with two other PD-linked loci – VPS35 (Vacuolar protein sorting 35), a component of the retromer complex involved in Golgi sorting, and the GTPase Rab7L (MacLeod *et al*, 2013). Both of these LRRK2 interactors are important trafficking proteins, further implicating LRRK2 (and more generally PD) in lysosome function.

Several other lysosomal genes are known to contribute to PD. For instance, mutations in *ATP13A2* (*PARK9*) cause the juvenile parkinsonian syndrome Kufor-Rakeb disease (Ramirez *et al*, 2006) and genome-wide association studies have identified *SCARB2* and *LAMP3* as PD-risk loci (Do *et al*, 2011; Nalls *et al*, 2011). These three PD genes encode lysosomal membrane proteins. Notably, the protein encoded by *SCARB2* (LIMP2; lysosomal integral membrane protein 2) is involved in the trafficking of glucocerebrosidase to the lysosomes (Reczek *et al*, 2007).

Mutations in *GBA1*, *LRRK2* and other lysosomal genes therefore provide compelling evidence that lysosomal dysfunction might play a primary pathogenic role in PD.

5 Lysosomal Ca^{2+} signalling in Parkinson disease – making the connection

If Ca^{2+} homeostasis and more “traditional” lysosomal functions, like autophagy and trafficking, are impaired in PD it begs the question whether lysosomal Ca^{2+} signalling is also disrupted. Recently, several studies have identified lysosomal Ca^{2+} signalling defects in PD.

5.1 GBA1-mediated Parkinson disease

To date, lysosomal Ca^{2+} signalling defects have only been reported in a handful of diseases (reviewed in Morgan *et al*, 2011). Many of these are lysosomal storage disorders (LSDs). For instance in NPC, an LSD characterised by the degeneration of cerebellar neurons, lysosomal Ca^{2+} content is reduced (Lloyd-Evans *et al*, 2008). This reduction was associated with impaired NAADP-evoked Ca^{2+} signalling and vesicular trafficking. GD, caused by homozygous mutations in *GBA1*, is another LSD and although ER (Korkotian *et al*, 1999;

Lloyd-Evans *et al*, 2003; Pelled *et al*, 2005) and agonist-evoked (Westbroek *et al*, 2016) Ca^{2+} signalling defects have been reported in various GD models, lysosomal Ca^{2+} had not previously been examined. Therefore, we sought to measure lysosomal Ca^{2+} signalling in GD and PD patients with *GBA1* mutations using the lysosomotropic agent GPN (Kilpatrick *et al*, 2016a). Under ER blockade, necessary to functionally dissect lysosomes from the ER (Kilpatrick *et al*, 2013), GD and *GBA1*-PD fibroblasts exhibited reduced global Ca^{2+} responses to direct lysosomal mobilisation by GPN when compared to aged-matched healthy cells. This is indicative of lowered lysosomal Ca^{2+} content. Furthermore, using various lysosomal markers, we noted that lysosomes appeared enlarged and clustered in GD and *GBA1*-PD. It is notable that a recent study reported a similar disruption to lysosome morphology in *GBA1*-PD patient derived iPSC (induced pluripotent stem cell) neurons (Schöndorf *et al*, 2014).

Apart from our assessment of lysosome well-being, we showed that ER Ca^{2+} content in fibroblasts from healthy individuals was increased in an age-dependent manner (Kilpatrick *et al*, 2016a). In younger GD and *GBA1*-PD patients, ER Ca^{2+} content was higher relative to aged-matched controls and thus resembled ER Ca^{2+} of older individuals. Moreover, enhanced ER Ca^{2+} content was associated with increased signalling through ER Ca^{2+} channels. This data compliments another study identifying increased ER Ca^{2+} signalling in *GBA1*-PD (Schöndorf *et al*, 2014). We extended our findings to show that dysfunctional ER Ca^{2+} signalling was a unique feature of disease since ER Ca^{2+} signals in asymptomatic *GBA1* carriers were unchanged. Therefore, our study identifies reciprocal remodelling of Ca^{2+} stores by pathogenic *GBA1* which may predispose individuals to PD. Due to the functional coupling between lysosomes and the ER, perhaps the Ca^{2+} levels within these stores equilibrate as a form of compensation. However, further work is needed to uncover which Ca^{2+} store is origin of the dysfunction and why.

It is intriguing that, like in PD, dihydropyridines (L-type VGCCs antagonists) can partially recover GD pathologies, namely restoring glucocerebrosidase enzyme activity. It has been suggested that dihydropyridines either act directly as pharmacological chaperones (Rigat & Mahuran, 2009) or by elevating ER Ca^{2+} levels (indirectly through inhibiting VGCC-evoked Ca^{2+} -induced Ca^{2+} release from the ER, Mu *et al*, 2008) which increases the activity of Ca^{2+} -regulated protein chaperones (Ong *et al*, 2010; Wang *et al*, 2011). Whichever way, mutated glucocerebrosidase can be correctly folded and trafficked to the lysosomes where it can resume its degradative activity. Whether VGCCs antagonists improve Ca^{2+} signalling and enzyme folding in *GBA1*-PD remains to be investigated. A recent study established that dihydropyridines additionally block TPCs, this conserved pharmacology is likely a consequence of TPCs being evolutionary ancestors of VGCCs (Rahman *et al*, 2014). Perhaps the potential efficacy of these drugs in PD/GD is a result of their targeting of multiple Ca^{2+} channels and stores. These studies further validate the targeting of VGCCs in PD.

To summarise, lysosomal Ca^{2+} content is reduced in *GBA1*-mediated PD and associated with disrupted endo-lysosomal morphology. Although further work is required to uncover the cause of lysosomal dysfunction, targeting Ca^{2+} channels might be therapeutic.

5.2 LRRK2-mediated Parkinson disease

In addition to *GBA1*-mediated PD, research also suggests that *LRRK2*-PD is associated with deregulated lysosomal Ca^{2+} signalling. As mentioned above, *LRRK2* is a common genetic cause of PD. The prevalent mutation, G2019S, targets the kinase domain of *LRRK2* and increases catalytic activity (West *et al*, 2005). Although the downstream consequences of this gain-of-function are largely unknown, the endo-lysosomal system appears to be involved (Biskup *et al*, 2006; Alegre-Abarrategui *et al*, 2009).

Gómez-Suaga *et al*, (2012) were the first to connect lysosomal Ca^{2+} signalling to *LRRK2*-PD. Initially, they found that the induction of autophagy was increased in cells overexpressing wild-type and mutant (G2019S) *LRRK2*. Such increases were attributed to Ca^{2+} -dependent activation of CaMKK- β /AMPK (calcium-dependent protein kinase kinase- β /adenosine monophosphate (AMP)-activated protein kinase, known inducers of the autophagic pathway) since they were abolished through Ca^{2+} chelation. Given that both lysosomes and Ca^{2+} are established regulators of autophagy (Høyer-Hansen *et al*, 2007) the authors reasoned that *LRRK2*-dependent autophagic defects could be reversed by targeting lysosomal Ca^{2+} . Accordingly, antagonising NAADP signalling or overexpressing dominant-negative TPC2 corrected pathology. The Hilfiker laboratory extended these results to show that TPC2 and *LRRK2* co-immunoprecipitate, which suggests that TPCs are a direct target for *LRRK2*. The recent finding that *LRRK2* also associates with VGCCs (specifically $\text{Ca}_v2.1$, Bedford *et al*, 2016) poses some intriguing questions – are ion channels a primary target for *LRRK2*? Might *LRRK2* affect $\text{Ca}_v1.3$ function also?

Gómez-Suaga and colleagues (2012) implicate *LRRK2* in hyperactive lysosomal Ca^{2+} signalling. However, since their study relied on *LRRK2* overexpression, and similar defects were noted in pathogenic and wild-type *LRRK2* expressing cells, it is possible that the effects are not related to PD. So, in a separate study, we specifically examined lysosomal Ca^{2+} signalling in *LRRK2*-PD patient-derived fibroblasts with G2019S mutations (Hockey *et al*, 2015). We found that cytosolic Ca^{2+} responses after microinjecting NAADP were indeed increased in *LRRK2*-PD patients compared to age matched healthy controls. Furthermore, like *GBA1*-mediated PD, we identified extensive lysosomal morphology defects. A similar morphological phenotype has also been observed in neurons (MacLeod *et al*, 2006) and astrocytes (Henry *et al*, 2015) overexpressing mutant *LRRK2*. We found that lysosomal defects could be reversed by targeting TPCs. Specifically, deficiencies were corrected by pharmacologically inhibiting regulators (NAADP, Rab7 and $\text{PI}(3,5)\text{P}_2$) of TPCs and silencing TPC2 (but not TPC1). The selectivity between TPC isoforms is intriguing and could be a consequence of previously reported *LRRK2*-TPC2 interactions (Gómez-Suaga *et al*, 2012). Thus, our results are consistent with the hypothesis that pathological *LRRK2* over-activates TPC2 to cause dysfunction.

Like TPCs, a recent study has established that *LRRK2* overexpression increases VGCC activity (Bedford *et al*, 2016). Such excessive flux through Ca^{2+} channels, presumably places additional strain on the delicately balanced cellular Ca^{2+} network in SNc neurons. Various mechanisms exist for clearing Ca^{2+} from the cytosol, including mitochondrial buffering and $\text{Na}^+/\text{Ca}^{2+}$ exchange at the plasma membrane (Berridge *et al*, 2000). Recent studies have shown that *LRRK2* also influences these processes (Cherra *et al*, 2013; Yan *et*

et al., 2015). Therefore, LRRK2 appears to have widespread influence over the Ca^{2+} signalling toolkit.

In short, pathological LRRK2 increases NAADP-evoked lysosomal Ca^{2+} signals, likely through a direct interaction with TPC2, to disrupt endo-lysosomal morphology.

6 Concluding remarks

The lysosome is an integral Ca^{2+} store that, through local and global signalling, influences various cellular functions. Individually, Ca^{2+} signalling and lysosomes have been implicated in PD pathology. Now recent studies (discussed herein) suggest that lysosomal Ca^{2+} signalling is deregulated in this common neurodegenerative disorder.

Specifically, it has been reported that lysosomal Ca^{2+} levels are reduced in *GBA1*-mediated PD (Kilpatrick *et al.*, 2016a). Although not extensively examined, this phenotype is common to other neurodegenerative diseases. Notably, the Ca^{2+} content of lysosomes is also reduced in both NPC1 (Lloyd-Evans *et al.*, 2008) and familial Alzheimer disease (Coen *et al.*, 2012; Lee *et al.*, 2015a). It has been suggested that excessive activation of TRPML channels, caused by changes in lysosomal pH, depletes lysosomal Ca^{2+} in familial Alzheimer disease (Lee *et al.*, 2015). Perhaps, the increased NAADP-evoked Ca^{2+} signals measured in *LRRK2*-mediated PD (Hockey *et al.*, 2015) also drain the lysosomes of Ca^{2+} . However, the effects of LRRK2 on total lysosomal Ca^{2+} levels need to be measured. Likewise, whether the lowered lysosomal Ca^{2+} content in *GBA1*-mediated PD is caused by excessive Ca^{2+} signalling must also be examined.

The studies discussed in this review raise a key question – how does impaired lysosomal Ca^{2+} signalling contribute to PD? It is likely that multiple cellular functions become deregulated. For instance, as trafficking hubs, aberrant lysosomal Ca^{2+} channel activity might affect Ca^{2+} -dependent fusion events within the endo-lysosomal system. This could account for the disrupted endo-lysosomal morphology we identify in both *GBA1*- and *LRRK2*-PD. Similarly, the fusion of autophagosomes with lysosomes requires lysosomal Ca^{2+} signalling (Pereira *et al.*, 2011). Perhaps the autophagy defects profusely reported in PD (reviewed in Zhang *et al.*, 2015) are caused by disrupted lysosomal Ca^{2+} signalling, however further work is required to substantiate this. Additionally, impaired lysosomal Ca^{2+} levels might disrupt the delicately balanced cellular Ca^{2+} network. In SNc neurons, where excessive Ca^{2+} signals already cause stress, this could worsen the burden and trigger PD.

Given that lysosomal Ca^{2+} is disrupted in PD, these Ca^{2+} stores could represent a new therapeutic target. Recent studies have progressed our understanding of TPC pharmacology (Rahman *et al.*, 2014; Patel, 2015). Developing effective TPC antagonists, could not only have potential value for PD but also Ebola infection (Sakurai *et al.*, 2015), cancer (Favia *et al.*, 2016; Nguyen *et al.*, 2017) and cardiac injury (Davidson *et al.*, 2015), all of which have been linked to TPCs. Furthermore, significant advances have been made in the targeting of TRPMLs now that synthetic agonists (Shen *et al.*, 2012; Chen *et al.*, 2014) and antagonists (Samie *et al.*, 2013) have been identified. Since enhancing TRPML activity has been shown to reverse trafficking defects in various diseases (including MLIV; Chen *et al.*, 2014 and

NPC; Shen *et al.*, 2012) it is possible that similar treatments could additionally correct trafficking in PD.

Even though it is clear that lysosomal Ca^{2+} signalling is deregulated in several diseases and the pharmacology of lysosomal ion channels is emerging, we have much to do to improve our understanding of these Ca^{2+} stores. Essentially, we must develop a comprehensive molecular description of the components that regulate lysosomal Ca^{2+} to effectively target these stores in disease. These are our next challenges.

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Abbreviations

AMPK	Adenosine monophosphate (AMP)-activated protein kinase
α-syn	Alpha-synuclein
CaMKK	Calcium-dependent protein kinase kinase- β
CAX	$\text{Ca}^{2+}/\text{H}^{+}$ exchanger
ER	Endoplasmic reticulum
GD	Gaucher disease
GPN	Glycyl-L-phenylalanine 2-naphthylamide
iPSC	Induced pluripotent stem cell
LIMP2	Lysosomal integral membrane protein 2
LRRK2	Leucine-rich repeat kinase 2
LSD	Lysosomal storage disorder
MLIV	Mucopolidosis IV
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NPC	Neimann-Pick disease type C
NAADP	Nicotinic Acid Adenine Dinucleotide Phosphate
PD	Parkinson disease
PI(3,5)P₂	Phosphatidylinositol 3,5-bisphosphate
SNc	Substantia nigra pars compacta
TRP	Transient receptor potential
TRPML	Transient receptor potential mucolipins

TPC	Two-pore Channels
VPS35	Vacuolar protein sorting 35
VGCC	Voltage gated Ca^{2+} channel

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Figure 1. Connections between the lysosomal Ca^{2+} signalling apparatus and PD.

Lysosomes are acidic organelles bound by a single membrane expressing proteins such as LIMP2, LAMP3, ATP13A2 and NPC1. As the degradative compartments of the cell, these organelles degrade various molecules including α -syn. In particular, the enzyme glucocerebrosidase cleaves glucocerebroside to ceramide and glucose. Lysosomes contain other lytic enzymes, such as cathepsin C which, among other substrates, hydrolyses the lysosomal-permeabilising agent GPN, a commonly used lysosomotropic agent. Lysosomes are also important Ca^{2+} stores that release Ca^{2+} through multiple channels, including TPCs, TRPMLs, TRPM2, P2X4 and VGCCs. Some of these receptors can be activated by NAADP (Ca^{2+} mobilising messenger) and $PI(3,5)P_2$ (phosphoinositide). Furthermore, TPCs can be inhibited by dihydropyridines (L-type VGCC antagonists) and have been shown to associate

with the multi-domain enzyme LRRK2. The mechanism of Ca^{2+} uptake into the lysosomes is unknown, but it has been proposed that $\text{Ca}^{2+}/\text{H}^{+}$ exchangers and Ca^{2+} ATPases are required. Lysosomes are involved in numerous cellular functions and recent research highlights a central role for these organelles in the pathogenesis of PD. Mutations in lysosomal proteins associated with PD are highlighted in orange.