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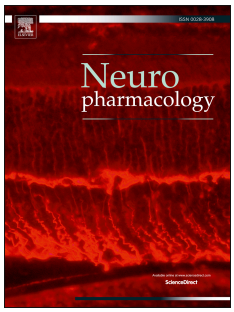

Ghrelin mediated neuroprotection - A possible therapy for Parkinson's disease?

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Abstract

Parkinson's disease is a common age-related neurodegenerative disorder affecting 10 million people worldwide, but the mechanisms underlying its pathogenesis are still unclear. The disease is characterised by dopamine nerve cell loss in the mid-brain and intra-cellular accumulation of α-synuclein that results in motor and non-motor dysfunction. In this review, we discuss the neuroprotective effects of the stomach hormone, ghrelin, in models of Parkinson's disease. Recent findings suggest that it may modulate mitochondrial function and autophagic clearance of impaired organelle in response to changes in cellular energy balance. We consider the putative cellular mechanisms underlying ghrelin-action and the possible role of ghrelin mimetics in slowing or preventing Parkinson's disease progression.

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(AB), autophagy-related (ATG), blood brain barrier (BBB), brain derived neurotrophic factor (BDNF), calorie restriction (CR), Ca^{2+}/calmodulin-dependent protein kinase (CAMK), cAMP response element binding protein (CREB), dentate gyrus (DG), diet-induced obesity (DIO), early growth response 1 (Egr-1), Forkhead box (FOXO), Glucagon-like peptide-1 (GLP-1), Growth hormone (GH), growth hormone secretagogue receptor (GHSR), High Temperature Requirement Protein A2 (HTRA2), Huntington's Disease (HD), Leucine-rich repeat kinase 2 (LRRK2), long term potentiation (LTP), mechanistic target of rapamycin (mTOR), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Microtubule Associated Protein 1 Light Chain 3 (LC-3), mitochondrial fusion protein (MFN), Neural stem cell (NSC), nicotinamide adenine dinucleotide (NAD^+), Nuclear factor-like 2 (NRF-2), Parkinson's disease (PD), proliferator-activated receptor γ coactivator 1α (PGC-1α), Protein Deglycase DJ-1, PTEN-induced putative kinase 1 (PINK1), reactive oxygen species (ROS), sirtuin (SIRT), superoxide dismutase 2 (SOD2), tubular sclerosis complex I (TSC), Unc-51 Like Autophagy Activating Kinase (ULK).

cell loss in the substantia nigra pars compacta (SNpc) and intracellular aggregation of α-synuclein. The symptoms involve a debilitating progressive decline in motor function that includes resting tremor, bradykinesia, rigidity and postural instability. Dopamine replacement therapies (i.e. L-DOPA) provide symptomatic relief, however, their prolonged use often contributes to severe motor side effects or dyskinesia. As current treatments for PD are ineffective in slowing disease progression there is an urgent need for alternative therapies. Thus, any new treatment that is successful in delaying the onset or slowing the progression of PD would provide significant health, social and economic benefit.

Calorie restriction (CR) in the absence of malnutrition has been linked to reducing the incidence of several neurodegenerative diseases, including PD (Maalouf, Rho, & Mattson, 2008). Conversely, diet-induced obesity increases the loss of dopaminergic cells in murine models of PD (Choi, Jang, Park, & Kang, 2005) and, although controversial, there is some evidence in humans that diet-induced obesity predisposes to PD (Hu, Jousilahti, & Nissinen, 2006). Whilst the mechanisms underlying this effect are not clear, they may be mediated by the gastrointestinal hormone, ghrelin, that is secreted from the stomach during CR to alert the brain to changes in metabolic status and to promote re-feeding (Kojima et al., 1999; Nakazato et al., 2001). In this review, we discuss the cellular and molecular mechanisms linked to ghrelin signalling in the CNS, with a focus on pathways associated with neurodegeneration in PD.

Gut-brain signaling of energy deficit

Ghrelin is a 28 amino-acid hormone that is elevated during CR (Bayliss et al., 2016; Lutter et al., 2008), a unique response as other gastrointestinal hormones are elevated by feeding to induce satiety. Activation of the ghrelin gene leads to the transcription and translation of the preproghrelin peptide that is subsequently cleaved into two known products - unacylated ghrelin (UAG) and obestatin. UAG can undergo post-translational acylation by ghrelin o-acyltransferase (GOAT) to form acyl-ghrelin (AG) (Gutierrez et al., 2008; Yang, Brown, Liang, Grishin, & Goldstein, 2008). UAG accounts for ~80% of circulating ghrelin. Work investigating UAG and AG suggest that AG has neuroprotective properties that UAG lacks (Bayliss et al., 2016). Therefore, elevating the ratio of circulating AG:UAG may have therapeutic application in slowing, preventing or reversing neurodegeneration. AG is believed to exert its neuroprotective effects directly in the brain by crossing the blood brain barrier (BBB). The acylation of ghrelin contributes to the specificity of its transport across the

(Kojima et al., 1999) which is expressed in several brain regions (Diano et al., 2006; Mani et al., 2017; Zigman, Jones, Lee, Saper, & Elmquist, 2006). GHSR is a member of the rhodopsin-like family of GPCRs that is expressed widely in the body, including the stomach, muscle, and brain (Y. Sun, Garcia, & Smith, 2007)(REF). A GHSR-eGFP reporter mouse (Mani et al., 2014) shows high levels of GHSR expression in mature neurones of the hippocampus, including dentate gyrus and CA1 (Hornsby et al., 2016), with moderate levels of expression within the SNpc. There is evidence of peripheral ghrelin binding to GHSR within the hippocampus (dentate gyrus, CA1, CA3), ventral tegmental area (VTA) and SNpc (Abizaid & Liu, 2006; Cabral, Fernandez, & Perello, 2013; Diano et al., 2006; Palotai et al., 2013), supporting the notion that ghrelin is acting directly in the brain.

Obestatin is reported to improve memory function (Carlini, Schiöth, & Debarioglio, 2007; Koyuncuoğlu et al., 2017) and protect neurones in a model of AD (Gargantini et al., 2016). However, obestatin's effect on GH secretion (Bresciani et al., 2006) and ingestive metabolites (Zhang et al., 2005) are opposite to that of ghrelin. As obestatin does not cross the BBB (Pan, Tu, & Kastin, 2006) the mechanism of obestatin-induced neuroprotection is likely distinct from AG. Notably, in the lactacystin model of PD, CR-mediated neuroprotection is seemingly independent of GHSR (Coppens et al., 2017) - obestatin may play a role in this paradigm.

There is increasing evidence linking ghrelin to PD. Firstly, plasma ghrelin levels are decreased in patients with PD (Fiszer et al., 2010) and their ghrelin response to food seems to be impaired (Song et al., 2017), suggesting that the ghrelin pathway is dysregulated in PD. Importantly, both ghrelin and GHSR gene products protect against the loss of SNpc neurones in the mouse MPTP model of PD (Andrews et al., 2009) and exogenous administration of acyl-ghrelin protects against neurodegeneration in rodent models of PD (Andrews et al., 2009; J. Bayliss et al., 2016; Jiang, Li, Wang, & Xie, 2008; Moon et al., 2009). In addition, other cellular events are triggered by administration of exogenous acyl-ghrelin, including increased adult hippocampal neurogenesis (Kent et al., 2015) and an increase in the number of spine synapses in the CA1 region of the hippocampus in wild-type mice and restoration of reduced spine number detected in ghrelin$^{-/-}$ mice (Diano et al., 2006). Acyl- ghrelin bound to neurones within the VTA and cortical neurones contributes to increased neuronal activity, dopamine turnover and synapse formation (Abizaid & Liu, 2006; Stoyanova, le Feber, & Rutten, 2013). These events may play a role in acyl-ghrelin-

Ghrelin's mechanisms of action

In neurones, acyl-ghrelin upregulates the calcium/calmodulin-dependent protein kinase-B (CAMK), which activates AMPK (Anderson et al., 2008; Bayliss et al., 2016; Chen et al., 2011). More specifically, ghrelin can increase AMPK activity in the hypothalamus (Andrews et al., 2008) and in SNpc DA neurones *in-vitro* (Bayliss et al., 2016). Indeed, AMPK is essential for acyl-ghrelin-mediated neuroprotection in the mouse MPTP model of PD (Bayliss et al., 2016). Together, these findings demonstrate that acyl-ghrelin regulates AMPK function to promote neuronal survival. The precise mechanism linking AMPK signals to neuroprotection is unclear, however, SIRT-family proteins are also increased following CR within the brain (Cohen et al., 2004; Crujeiras, Parra, Goyenechea, & Martínez, 2008; Yu, Zhou, Lin, Fu, & Wang, 2014). Indeed, the anti-aging effects of CR are dependent on ghrelin-agonist activation of SIRT1 (Fujitsuka et al., 2016) and in whole brain tissue, SIRT1 expression requires the activation of the ghrelin receptor (Yang et al., 2016). SIRT1 is also required for the orexigenic effects of ghrelin (Velásquez et al., 2011). As the induction of AMPK during CR also leads to the upregulation and activity of SIRTs (Canto et al., 2009; Cohen et al., 2004) this suggests a possible link between CR, ghrelin, AMPK, SIRTs and neuroprotection.

CR and/or acyl-ghrelin may also protect neurones by inhibiting the activity of mTOR. Indeed, mTOR activity is decreased during CR and is reported to improve the symptoms of age-related neurodegenerative disease (Dong et al., 2015; Yang et al., 2014). Notably, a comparison of the hippocampus from control and AD patients showed that several proteins of the mTOR pathway were upregulated in patients with severe AD (Sun et al., 2014). These data support a mechanism involving CR/Ghrelin/CAMK/AMPK/mTOR in both non-neuronal (Ghislat, Patrons, Rizzutos, & Knecht, 2012; Mao et al., 2015) and neuronal cell models (Bayliss et al., 2016).

*Mitochondria***:** The underlying cause of PD is unknown, however, mitochondrial dysfunction and/or damage is thought to contribute to its pathophysiology (Abou-Sleiman et al., 2006; Hawong, Patterson, Winner, Goudreau, & Lookingland, 2015; Kitada et al., 1998; Schapira et al., 1989; Sorrentino et al., 2017). Mitochondrial malfunction can lead to reduced mitochondrial biogenesis (St-Pierre et al., 2006), increased synthesis of free radicals and oxidative stress (Lin & Beal, 2006; Perfeito, Ribeiro, & Rego, 2016; St-Pierre et al., 2006), decreased production of ATP (Mann et al., 1992), build-up of H^+ ions due to reduced mitochondrial uncoupling (Andrews et al., 2009; Conti et al., 2005) and deficient calcium

including α-synuclein (Krüger et al., 1998; Polymeropoulos et al., 1997; Singleton, 2003; Zarranz et al., 2004), parkin (Kitada et al., 1998), leucine-rich repeat kinase 2 (LRRK2) (Simón-sánchez et al., 2009; Zimprich et al., 2004), HTRA2 (also called OMI) (Strauss et al., 2005), PTEN-induced putative kinase 1 (PINK1) (Abou-Sleiman et al., 2006; Gandhi, 2006; Puschmann et al., 2017; Valente et al., 2004), DJ1 (Bonifati, 2003; Bonifati et al., 2003; Hague, Rogaeva, Hernandez, & C, 2003; Lockhart, 2004) and mitochondrial complex I (Schapira et al., 1990, 1989).

Mitochondrial fission/fusion: Changes in cellular energy demands or oxidative damage lead to alterations in mitochondrial shape through a process of fission and fusion (Baloh, Schmidt, Pestronk, & Milbrandt, 2007; Hoppins, Lackner, & Nunnari, 2007). The rate of fission and fusion is continually changed in response to the variable energy demands of the cell (Detmer & Chan, 2007). Notably, these processes may be impaired during PD. In cell models of PD, mutated α-synuclein triggers an increase in mitochondrial fission and prevents fusion (Guardia-Laguarta et al., 2014; Kamp et al., 2010; Nakamura et al., 2011) whereas wild type α-synuclein protects the shape of neuronal mitochondria against damage from oxidative stress and neurotoxins (rotenone and 6-hydroxydopamine) (Menges et al., 2017). Indeed, morphological changes induced by overexpression of mutated α-synuclein are prevented by the PD related proteins Parkin, PINK-1 and DJ-1 (Kamp et al., 2010).

Mild stress, such as energy deficiency or short term nutrient deprivation cause mitochondria to fuse thereby boosting energy production (Rambold, Kostelecky, Elia, & Lippincott-Schwartz, 2011). However, severe stress such as starvation (or absence of glucose), increases the production of ROS causing damage to proteins, lipids and DNA as well as impairing ATP synthesis and causing mitochondrial fission (Rambold et al., 2011; Rossignol et al., 2004; H. Zhang et al., 2016). Interestingly, fission can be directly induced by AMPK activation can directly trigger mitochondrial fission and fission induced by the electron-transport-chain (ETC) inhibitor, rotenone, is attenuated in the absence of AMPK (Toyama et al., 2016). Therefore, whilst there is no direct evidence linking acyl-ghrelin to changes in neuronal mitochondrial fission/fusion dynamics, we suggest that this pathway should be investigated given the AMPK-dependent neuroprotective effects of acyl-ghrelin.

Mitochondrial fragmentation/fission is dependent on the recruitment of dynamin-related protein 1 (DRP1) (also called dynamin-like protein 1 (DLP1)) from the cytoplasm to the fission locus on the mitochondrial membrane. Notably, DRP1 gene deletion in mice causes

Wang et al., 2008). In one study, inhibition of DRP1 decreased mitochondrial fragmentation, reduced the loss of mitochondrial membrane potential and improved cognitive function (Baek et al., 2017). The formation of mitospheres, a term used to describe hyperpolarized and spherical mitochondria, is dependent on DRP1. DRP1 is important for dopamine neurone terminals, its blockade restores synaptic function when inhibited by ß-amyloid in an AD model (Baek et al., 2017). Indeed, there are multiple studies supporting a link between DRP1 and PD. For example, the inhibition of DRP1 protects murine dopaminergic neurones from MPTP damage (Filichia, Hoffer, Qi, & Luo, 2016) and the loss of DRP1 prevents accumulation of α-synuclein (Berthet et al., 2014). However, the loss of DRP1 and α-synuclein is followed by a decrease in mitochondrial respiration and an increase in the degradation of neurones, demonstrating a regulatory role in basal neuronal function (Berthet et al., 2014). These reports indicate a link between the two proteins, with a DRP1 dependent mechanism that leads to mitochondrial fragmentation. In humans, reduced DRP1 expression is reported in the SNpc and astrocytes of PD patients (Hoekstra et al., 2014; Jin et al., 2006). It should be noted that α-synuclein can cause the fragmentation of mitochondria directly, without the requirement for DRP1 (Nakamura et al., 2011). Further studies are required to define the cellular pathways that link DRP1, α-synuclein and mitochondrial fragmentation in the context of PD. Notably, CR (40% CR over 6 or 18 months) increases the number, size and surface area of mitochondria in the liver, and include an increase in the fission related proteins DRP1 and Fis1 in mice (Khraiwesh et al., 2013). DRP1 is also increased in the brain homogenates of mice treated with DNP, a mitochondrial uncoupler that mimics the effects of CR (Liu et al., 2015). These data suggest that nutrient restriction, and possibly acyl-ghrelin, may regulate mitochondrial dynamics. However, further work is needed to determine whether these effects are mediated via acyl-ghrelin and whether they are linked to ghrelin-mediated protection of mid-brain dopaminergic neurones.

Mitochondrial biogenesis: Mitochondrial biogenesis is another aspect of mitochondrial function that is altered by CR (López-Lluch et al., 2006) and is linked to neuronal function and longevity. In particular, CR can halt the dysfunction in age-associated mitochondrial bioenergetics as well as increasing mitochondrial biogenesis in healthy individuals (Civitarese et al., 2007; López-Lluch et al., 2006; Picca et al., 2013). Indeed, CR improves neuronal respiratory rates as well as survival (Cerqueira, Cunha, Laurindo, & Kowaltowski, 2012). Notably, ghrelin promotes an increase in neurone mitochondrial number and

mitochondrial biogenesis in mice and cell lines by altering the activity of proliferator-activated receptor γ coactivator 1α (PGC-1α) a master regulator of mitochondrial biogenesis (Lagouge et al., 2006; Nemoto, Fergusson, & Finkel, 2005; Rodgers et al., 2005). PGC-1α is implicated in PD with PGC-1α null mice being more prone to neuronal damage following treatment with the electron transport chain inhibitor, MPTP (St-Pierre et al., 2006). Conversley, activation of PGC-1α inhibits dopamine neurone loss triggered by mutant α-synuclein or rotenone in cellular disease models (Zheng et al., 2010). Parkin, another mitochondrial regulator implicated in PD interacts with PGC-1α to preserve mitochondria and protect dopaminergic neurones (Zheng et al., 2017). Moreover, PCG-1α levels have been shown to increase following CR (Chiba et al., 2009) or fasting (Ding, Lichti, Kim, Gonzalez, & Staudinger, 2006). Furthermore, treadmill exercise improved mitochondrial function and prevented neuronal loss by reducing α-synuclein in a mechanism that involves both SIRT1 and PGC-1α (Koo & Cho, 2017; Koo, Cho, & Lee, 2017). These data raise the possibility that acyl-ghrelin activates PGC-1α via an AMPK-SIRT mechanism that results in enhanced resilience to neurotoxins.

Mitochondrial ROS: Mitochondria are dynamic organelles that play an important role in synaptic transmission (Ivannikov, Sugimori, & Llinás, 2013). Cycles of oxidative phosphorylation are carried out within the mitochondrial membranes that produce energy in the form of ATP that is required for cellular function. However, this also results in the formation of reactive oxygen species (ROS) that can damage cells. ROS are a combination of harmful compounds that include peroxides (H_2O_2), superoxide ($O^{\bullet}_2$), hydroxyl $OH^{\bullet}$ and oxygen radicals. They are typically generated by NADPH-dependent oxidases, within the mitochondria (predominantly complex I and/or complex III) and by various cellular enzymes involved in mitochondrial electron transport, B-oxidation, glycolysis and the TCA cycle (Liemburg-Apers, Willems, Koopman, & Grefte, 2015) as well as alternative oxidation reactions of fatty acids, lipids and eicosanoids. ROS are natural by-products of oxygen metabolism and also play a role in cell signalling and homeostasis (see reviews, (Finkel, 2011; Schieber & Chandel, 2014). However, ROS accumulation, can cause oxidative stress leading to mitochondrial dysfunction and impaired mitochondrial shape. Damaged mitochondria are generally fragmented, swollen and rounded (Ahmad et al., 2013), and mitochondria with damaged morphology have been identified in cases of PD (Baloyannis, Costa, & Baloyannis, 2006; Nakamura et al., 2011) and in PD models (Krebiehl et al., 2010;

ROS may be reduced in neurones by increasing the presence of ROS scavengers, such as citrate synthase and cytochrome-C (Cerqueira et al., 2012). Exercise reduces ROS production in murine models of PD and AD via a mechanism that is likely to involve SIRTs and PGC-1α (Cheng et al., 2016; Koo, Jang, et al., 2017; Koo, Kang, Oh, Yang, & Cho, 2017). Indeed, the SIRT activating compound Oligonol and exercise, increase levels of a mitochondrial antioxidant, superoxide dismutase 2 (Cheng et al., 2016; Park et al., 2016).
CR reduces oxidative damage to mitochondrial DNA in the liver (Lopez-Torres, Gredilla, Sanz, & Barja, 2002), possibly by reducing mitochondrial membrane potential (Chen et al., 2013). There is also evidence of CR and ghrelin decreasing ROS in several cell lines (Tong et al., 2012), murine liver mitochondria (Chen, Chen, Wang, & Li, 2012), skeletal muscle (Chen et al., 2014) and in particular, within mouse neurones (Andrews et al., 2008) and the rat brain (Sanz et al., 2005). Also, CR reduces ROS in mitochondria from whole mouse brain homogenates by inhibiting mTOR (Miwa et al., 2016). In neuronal synaptosomes (synaptic terminals isolated from neurones) ghrelin stimulates mitochondrial respiration and lowers ROS by increasing UCP2 (Andrews et al., 2008), a mitochondrial protein that transports protons with the aim of lowering the proton-motive force and attenuating the production of superoxide by the electron transport chain (Brand & Esteves, 2005). Importantly, this effect has been observed in hypothalamic synaptosomes. Furthermore, ghrelin prevents the loss of dopaminergic neurones from the SNpc and striatum in mice treatment with MPTP via a mechanism that is dependent on UCP2 (Andrews et al., 2009). These data provide significant support for ghrelin signalling ameliorating ROS-induced neurone damage in models of PD.

Autophagy: Damaged mitochondria must either be repaired or removed. Unresolved, mitochondria can contribute to neuronal damage and death. In particular, insufficient clearance of dysfunctional mitochondria has been associated with PD (Kitada et al., 1998; Youle, & van der Bliek, 2012). These studies have led to renewed efforts to improve our understanding of cellular energy regulation and to identify methods that promote mitochondrial function. For example, the removal of dysfunctional mitochondria via mitophagy (the specific removal and degradation of mitochondria by autophagy) is considered as a therapeutic target for the treatment of PD. Mitophagy is influenced by diet, as mice fed a high calorie diet have increased PINK1 protein expression (a marker of mitochondrial damage) leading to mitophagy induction in kidneys (Cui et al., 2013). Conversely, mice undergoing CR have a reduction in renal PINK1. There is evidence to

least in part, via mitophagy. However, at present, it is unclear whether CR similarly regulates mitophagy in midbrain neurones.

As mentioned above, autophagy is the process of removing damaged organelles and/or proteins within a cell. It is required for maintaining cellular nutrient homeostasis and is essential for life (Kuma et al., 2004). Autophagy is particularly important in the maintenance of healthy neurones with defective autophagy linked to neuronal death (Friedman et al., 2012; Larsen & Sulzer, 2002; Liberski, Gajdusek, & Brown, 2002). Notably, the autophagy pathway is down-regulated and negatively correlates with AD progression (Cecarini et al., 2014). Defects in autophagy are now becoming more commonly reported in a range of neurodegenerative disorders, including PD (Poehler et al., 2014), HD (Vidoni, Secomandi, Castiglioni, Melone, & Isidoro, 2017), prion diseases (Liberski et al., 2002) and lysosomal storage diseases (Vitner, Platt, & Futerman, 2010). As a result, there is significant interest in the role of autophagy in these diseases and how it could be targeted to identify new therapies for neurodegenerative diseases.

Autophagy and Parkinson's disease: Genetic analysis of patients with sporadic PD have revealed mutations in several genes, including ATG5 (Garcia-Garcia et al., 2013) and ATG7 (Chen et al., 2013), whose products are considered essential for the induction of autophagy. Furthermore, the expression of lysosome-associated membrane protein 2A (LAMP2A) is reduced in the SN of PD patients (Alvarez-Erviti et al., 2010). Importantly, LAMP2A is involved in chaperone-mediated autophagy and the degradation of α-synuclein in murine dopamine neurones (Tang et al., 2015). The association between autophagy and PD is further supported by several pre-clinical studies. For example, over-expression of α-synuclein, which is detrimental to rat cortical neurones (Wani et al., 2017), inhibits autophagy in an astrocyte cell line (Erustes et al., 2017). Mutant α-synuclein (A53T and A30P) impairs the efficacy of chaperone-mediated autophagy in rat neurones (Cuervo, 2004). However, enhancing autophagy with intra-SN Cystatin C treatment had a neuroprotective effect in the A53T α-synuclein transgenic mouse, with a reduction in α-synuclein and an increase in the autophagy protein, LC3B (Zou et al., 2017). Furthermore, silencing of PINK1 leads to impaired function of the autophagic protein, Beclin1 (Gelmetti et al., 2017). Whereas, PINK deficiency leads to neurone loss that can be prevented by the activation of autophagy (Zhang et al., 2017). These data suggest an important link between autophagic dysfunction and the occurrence of PD.

vary significantly in response to starvation conditions and CR (Mizushima, Yamamoto, Matsui, Yoshimori, & Ohsumi, 2004). In particular, short-term food deprivation (~1-4 hours) has been shown to increase the formation and/or accumulation of autophagosomes in mouse cortical neurones (Alirezaei et al., 2010) and neuronal cell lines (Kaushik et al., 2012).

Several regulators of the energy sensing network that are responsive to CR, including CAMK, AMPK and mTOR, have links with autophagy and PD. For example, the neuroprotective effects of CR-induced autophagy are dependent on AMPK (Hayakawa et al., 2013). AMPK increases autophagosome number in response to the PD related protein LRRK2 (Gómez-Suaga et al., 2012) and can activate autophagy directly by phosphorylating ULK, an autophagy-related protein (Kim, Kundu, Viollet, & Guan, 2011). In addition to the direct activation of autophagy by AMPK via ULK, it is likely that AMPK induced autophagy may involve SIRTs as amyloid-β can inhibit AMPK leading to the downregulation of SIRT1 and neuronal autophagy (Lin et al., 2016). Consistent with this finding, the SIRT1 activator, resveratrol, activates autophagy via AMPK and SIRT1 in neuronal models of PD (Wu et al., 2011). To add further complexity to the interaction between AMPK and SIRTs, both can have opposing effects on p53 (Vaziri et al., 2001) and autophagic clearance of aggregated α-synuclein may also involve SIRT2 (de Oliveira et al., 2017).

Distinct from the CAMK/AMPK/SIRT mechanism, autophagy can be induced via inhibition of mTOR. For example, deletion of the mTOR gene in a murine model of AD causes an increase in ATG3, ATG5, ATG7 and ATG12 (Caccamo, De Pinto, Messina, Branca, & Oddo, 2014). In cell and animal models of PD the over-expression of α-synuclein up-regulates mTOR and impairs autophagy (Erustes et al., 2017; Jiang et al., 2013; Wani et al., 2017). Furthermore, mutations in mouse tubular sclerosis complex I (TSC1) or TSC2 genes, which leads to increased mTOR activity (Inoki, Zhu, & Guan, 2003), reduces autophagy in both the hippocampus and cortex (McMahon et al., 2012). In addition, mTOR activity was decreased and autophagy increased in a study investigating effects of CR in mouse cortical and purkinje neurones (Alirezaei et al., 2010). The mitochondrial uncoupler, DNP, which mimics CR, suppresses mTOR leading to increased autophagy in mouse brain homogenates (Liu et al., 2015). Notably, ghrelin may induce autophagy by inhibiting mTOR (Mao et al., 2015). The inhibition of mTOR and activation of autophagy is also dependent on CAMK (Ghislat et al., 2012), which further supports the proposed mechanism described in Figure 1. Importantly, the ghrelin/mTOR/autophagy mechanism may be relevant in the context of PD,

neuronal models. For example, CR in acyl-ghrelin deficient (*Goat*$^{-/-}$) mice leads to a reduction in LC3-II in liver, suggesting a decreased rate of autophagy (Yuanyuan Zhang, Fang, Goldstein, Brown, & Zhao, 2015). The treatment of hepatic cells with ghrelin leads to an increase in LC3-II, ATG5, ATG7 as well as a decrease in p62 (Sqstm1), consistent with increased rates of autophagy (Ezquerro et al., 2016). Ghrelin increases LC3 and Beclin1 in an AMPK dependent manner in rat smooth muscle cells (Xu, Liu, Song, Chen, & Gui, 2017), and ghrelin-induced autophagy may occur via activation of AMPK to prevent lipotoxicity in human liver cells (Mao et al., 2015). Moreover, the activity of various lysosomal enzymes, which are required for the breakdown of autophagosomes is increased in the plasma of rabbits treated with ghrelin (Witek et al., 2005). Importantly, recent studies provide evidence that the neuroprotective properties of ghrelin may be mediated, at least in part, via autophagy. In particular, ghrelin decreased the activity of Cathepsin B in AD-like (SH-SY5Y) cells (Cecarini et al., 2014), a key protein in the autophagy pathway. While CR stimulated autophagy by activating ghrelin receptors in rat cortical neurones (Marques et al., 2016).

These data highlight CR- and ghrelin-mediated pathways that may lead to autophagy and neuroprotection. However, the precise molecular mechanism(s) underlying the ghrelin-autophagy pathway is not well understood in the context of PD. In particular, further studies are required to assess ghrelin-mediated autophagy in models of PD that involve the clearance of mis-folded proteins such as α-synuclein.

Drug repurposing for novel PD treatments

Acyl-ghrelin and synthetic mimetics represent putative therapeutic agents as they are likely to target pathways impaired in PD. Activation of such pathways may slow or prevent neurone loss in the SNpc and may ameliorate non-motor PD impairments, including, reduced gastric motility, weight loss and cognitive decline. Acyl-ghrelin could be administered directly to patients, however, its short half-life (~15 to 30 minutes) means that it would require repeated intravenous administration. A stable acyl-ghrelin mimetic that is orally available would be more appropriate.

Several such GHSR agonist compounds have been generated to treat endocrine and cancer-related disease. For example, adlumiz (anamorelin hydrochloride) developed by Helsinn Birtex Pharmaceuticals is a GHSR-agonist for the treatment of anorexia and cachexia in non-small cell lung cancer. The GHSR-agonist, Macimorelin (developed by

compounds have been shown to regulate CNS physiology. MK-0667 (ibutamoren mesylate) developed to treat muscle wasting and growth hormone deficiency, promotes the accumulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on mouse excitatory hippocampal synapses and enhances long term potentiation (LTP), a physical correlate of memory function (Ribeiro et al., 2014). In addition, peripheral administration of GSK894490A and CP464709-18 (developed by GSK) and LY444711 (developed by Eli Lilly) enhance learning and memory in rats (Atcha et al., 2009; Diano et al., 2006). However, a detailed study of whether they activate GHSR on mid-brain neurones along with analysis of any neuroprotective efficacy is currently lacking in diverse models of PD.

An alternative therapeutic approach would be to increase circulating acyl-ghrelin levels. Circulating endogenous acyl-ghrelin readily crosses the blood-brain-barrier (BBB) to promote adult hippocampal neurogenesis and learning (Hornsby et al., 2016), as well as confer protection on SNpc neurones (Bayliss et al., 2016). Therefore, increasing the bioavailability of acyl-ghrelin by preventing its enzymatic de-acylation should be considered as a possible therapeutic approach. Indeed, Acyl-Protein Thioesterase 1 (APT1), has been identified as a serine protease enzyme that de-acylates ghrelin (Satou, Nishi, Yoh, Hattori, & Sugimoto, 2010). As the structure of the APT1 active site has been resolved by X-ray crystallography this may provide an appropriate target for novel drug discovery (Devedjiev, Dauter, Kuznetsov, Jones, & Derewenda, 2000). In addition, butyrylcholinesterase has been identified as an alternative ghrelin de-acylating enzyme (Schopfer, Lockridge, & Brimijoin, 2015). Given that drugs such as rivastigmine, which is used for the treatment of mild to moderate dementia, effectively inhibit both butyrylcholinesterases and acetylcholinesterases (Nordberg, Ballard, Bullock, Darreh-Shori, & Somogyi, 2013), further analysis of these compounds are warranted in the context of acyl-ghrelin signalling.

In summary, we describe accumulating data that support a role for acyl-ghrelin in regulating mitochondrial function and autophagy to slow or even prevent neurodegeneration. Therefore, we suggest that drug-repurposing efforts are required to delineate the efficacy of GHSR-agonists in models of neurodegenerative disease. Additionally, establishing the underlying cellular and molecular mechanisms of acyl-ghrelin/GHSR signalling within the nervous system may identify novel pathways relevant to nerve cell survival in PD.

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Figure legend

Figure 1. Schematic representation of putative cellular mechanisms triggered by calorie restriction (CR) mediated activation of the ghrelin-signalling pathway. Ghrelin-GHSR signalling results in adaptations to mitochondria and autophagic flux, leading to neuroprotection. CR increases circulating acyl-ghrelin which crosses the blood brain barrier (BBB) and binds growth hormone secretagogue receptor (GHSR) leading to activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMK) and AMP-activated protein kinase (AMPK)-mediated autophagy by up-regulating sirtuins (SIRTS) or the formation of the Unc-51 Like Autophagy Activating Kinase (ULK) complex. In addition, AMPK may inhibit mechanistic target of rapamycin (mTOR) to induce autophagy, whilst promoting uncoupling protein 2 (UCP2) dependent changes in mitochondrial biogenesis. Abbreviations: autophagy-related (ATG), Leucine-rich repeat kinase 2 (LRRK2), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α).

Figure 1. Morgan *et al*.