Many of the neurodegenerative diseases that afflict people are caused by intracytoplasmic aggregate-prone proteins. These include Parkinson’s disease, tauopathies, and polyglutamine expansion diseases, like Huntington’s disease. In Mendelian forms of these diseases, the mutations generally confer toxic novel functions on the relevant proteins. Thus, one potential strategy for dealing with these mutant proteins is to enhance their degradation. This can be achieved by upregulating macroautophagy, which we will henceforth call autophagy. In this review, we will consider the reasons why autophagy upregulation may be a powerful strategy for these diseases. In addition, we will consider some of the drugs and associated signalling pathways that can be used to induce autophagy with these therapeutic aims in mind.

Intracellular protein aggregation in neurodegenerative diseases

Intracellular protein misfolding/aggregation are features of many late-onset neurodegenerative diseases, called proteinopathies. These include Alzheimer’s disease, Parkinson’s disease, tauopathies, and polyglutamine (polyQ) expansion diseases, such as Huntington’s disease (HD) and various spinocerebellar ataxias (SCAs), like SCA3 (1,2). Currently, there are no effective strategies to slow or prevent the neurodegeneration resulting from these diseases in humans.

All known polyQ mutant proteins form intracellular aggregates (inclusions) with amyloid-like structures in susceptible neurons (3). HD, the most prevalent of the nine polyQ expansion diseases, is caused by an abnormally expanded CAG trinucleotide repeat tract in the IT15 gene (>35 repeats). These repeats are translated into an elongated polyQ tract close to amino-terminus end of the huntingtin protein. Huntingtin is mainly cytosolic, but a small proportion is nuclear (4). In HD, intranuclear inclusions are seen in the rarer juvenile-onset cases, but extranuclear inclusions predominate in the more typical adult-onset cases. The causal role for inclusions in these diseases is debated, since some have reported dissociations between cell death and inclusion formation (4,5). Strong genetic and transgenic data argue that the primary consequence of the polyQ expansion mutations is to confer toxic gain-of-function on the mutant proteins (1,2,4,6). Indeed, a gain-of-function mechanism appears to underlie most of the Mendelian disorders caused by aggregate-prone proteins, including tauopathies and other polyQ expansion disorders. This does not exclude that the gain-of-function toxicity in diseases like HD may be modulated to some degree by loss-of-function effects, although transgenic data suggest that such putative effects are likely to be small (7). Since the mutations causing many proteinopathies (e.g., polyQ diseases and tauopathies) confer novel toxic functions on the specific proteins, and disease severity frequently correlates with expression levels, it is important to understand the factors regulating the synthesis and clearance of these aggregate-prone proteins.

Autophagic clearance of intracytosolic, aggregate-prone proteins

Our data suggest that accelerating the removal of toxic huntingtin fragments may be a tractable therapeutic strategy for HD (Fig. 1). We showed that the ubiquitin-proteasome and autophagy-lysosomal pathways are the major routes for mutant huntingtin fragment clearance (8). While the narrow proteasome barrel precludes entry of oligomers/aggregates of mutant huntingtin (or other aggregate-prone intracellular proteins), such substrates can be degraded efficiently by macroautophagy (which we will call autophagy).

Autophagy involves the formation of double-membrane isolation membranes called phagophores, which expand and engulf portions
of the cytoplasm forming double-membrane vesicles, called autophagosomes (9,10) (Fig. 1). Autophagosomes are formed randomly in the cytoplasm and then are trafficked along microtubules in a dynein-dependent fashion towards the microtubule organising centre, where they fuse with lysosomes forming autolysosomes, after which their contents are degraded (11,12). The only known mammalian protein that specifically associates with the autophagosome membrane (as opposed to other vesicles) is the microtubule-associated protein 1 light chain 3 (LC3), which is post-translationally modified into cytosolic LC3-I that conjugates with phosphatidylethanolamine upon autophagy induction, to form autophagosome-associated LC3-II (13).

Recent studies have shown that constitutive autophagy may play a pivotal role in the clearance of normally occurring cellular misfolded proteins, as loss of basal autophagy by conditional knockout of key autophagy genes, such as Atg5 or Atg7, in mouse brains resulted in a neurodegenerative phenotype and the formation of protein aggregates (14,15). We have shown that mutant huntingtin fragments, expanded polyalanines tagged to green fluorescent protein and mutant forms of α-synuclein (associated with forms of PD) are highly dependent on autophagy for their clearance in cell models (8,16). The clearance of these mutant proteins is delayed by autophagy inhibitors like 3-methyladenine (3-MA) and bafilomycin A1, or knockdown of autophagy genes, while autophagy induction with rapamycin enhances their clearance (8,17,18). Subsequently, other aggregate-prone proteins, like tau causing fronto-temporal dementias, mutant ataxin 3 associated with SCA3, mutant superoxide dismutase 1 (SOD1) associated with amyotrophic lateral sclerosis and mutant prion proteins causing prion diseases, have been shown to be autophagy substrates (19-22). While most of the wild-type counterparts of these mutant proteins are poor autophagy substrates, wild-type α-synuclein has been shown to be degraded by chaperone-mediated autophagy, a distinct lysosomal pathway (23). Thus, upregulating autophagy may be beneficial for the treatment of neurodegenerative diseases, and identification of autophagy enhancers could provide potential therapeutic candidates (Table 1) (24,25).

Chemical inhibitors of mTOR as autophagy inducers
At the time we did the first of these studies, the only drug that was known to induce autophagy that was in clinical use (for other indications) was rapamycin. Rapamycin is a highly specific inhibitor of the mammalian target of rapamycin (mTOR). The mTOR pathway, which is essential for controlling cell growth, protein synthesis, ribosome biogenesis, nutrient metabolism and autophagy, involves two functional complexes called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (26). In mammalian cells, rapamycin forms a complex with the immunophilin FK506-binding protein of 12 kDa (FKBP12), which binds to mTORC1 and inhibits its activity (27) (Fig. 2). However, recent studies have shown that prolonged treatment with rapamycin can inhibit mTORC2 activity in certain mammalian cell types (28,29). Recently, a selective ATP-competitive small molecule mTOR inhibitor called Torin1 has been found to induce autophagy to a much greater extent than rapamycin (30).

Recently, Roberge and colleagues reported a study where they screened a library of 3,500 chemicals with an automated cell-based assay to detect increases in autophagosome numbers (31). The screen identified four compounds, perhexiline, niclosamide, amiodarone and rottlerin, which stimulated autophagy by inhibiting mTORC1, but not mTORC2, signalling (Fig. 2). Rottlerin inhibited mTORC1 signalling via TSC2, while the other drugs inhibited mTORC1 signalling in a TSC2-independent manner (31). Interestingly, three of the identified compounds (amiodarone, perhexilene and niclosamide) are drugs already approved for other therapeutic indications, thereby reinforcing the rationale for targeting mTORC1 activity in diseases where positive modulation of autophagy may be beneficial.

Recent studies have identified some of the molecular components in mammalian autophagy downstream of mTORC1. Rapamycin appears to regulate mammalian autophagy by inhibiting the mTOR-mediated phosphorylation of Atg13 and ULK1, which are involved in autophagosome formation. This leads to dephosphorylation-dependent activation of ULK1 (and ULK2) and ULK1-mediated phosphorylation of Atg13, FIP200, and ULK1 itself, which triggers autophagy (Fig. 2). Thus, the ULK1-Atg13-FIP200 complex appears to have...
integrate the autophagy signals downstream of mTORC1 (32-34). However, it is not clear yet how phosphorylation of these proteins regulates their activities.

Subsequent studies have provided robust support for our assertions using genetic and chemical approaches and suggest that autophagy is important for clearance of mutant huntingtin fragments, at least as large as the first one-third of the protein as well as full-length mutant huntingtin, and that wild-type forms are far less dependent on autophagy for their clearance compared to the mutant forms (17,18,24,35,36). We showed that rapamycin attenuated mutant huntingtin fragment toxicity in cells, and in transgenic Drosophila and mouse models of HD (37). The protective effects of rapamycin was blocked in flies expressing mutant polyalanines or expanded polyQ when the expression of different autophagy genes was reduced (20,38), suggesting that the major benefits of this drug were autophagy-dependent and were not mediated by alternative mechanisms, like impaired translation (at least in these in vivo settings). Our data in cell and fly models show that rapamycin-mediated autophagy upregulation may be valuable for many other intracellular proteinopathies, including SCA3, and both mutant and wild-type tau (20). Tau was of particular interest as it is mutated in certain fronto-temporal dementias, and wild-type tau is the major component of the neurofibrillary tangles that are believed to contribute to pathology in sporadic Alzheimer’s disease (20). Furthermore, elevated intracellular glucose or glucose-6-phosphate also induce autophagy by inhibiting mTOR (39) (Fig. 2).

An additional benefit of autophagy upregulation in these diseases is that it appears to protect cells against apoptotic insults (40). Thus, enhancing autophagy may have two beneficial effects in the context of neurodegenerative diseases. Firstly, it enhances removal of the toxic, aggregate-prone protein, and secondly, it protects cells from apoptosis.

The autosomal-dominant proteinopathies that are potentially amenable to autophagy upregulation present an important opportunity for delaying the onset of disease. Most patients will have a positive family history, and thus it is possible to identify most cases at risk of developing disease with a simple genetic test (4). Ideally, one would like to start treatment at the earliest possible age in such individuals to aim to delay the onset of disease. For instance, in HD, one would aim to delay onset from a median age of 40 until after normal life expectancy, and thus effectively prevent the disease.

One issue that remains unresolved is whether long-term autophagy upregulation may have deleterious effects. It is important to point out that our mouse studies involved rapamycin administration regimes that were pulsatile (37), and thus it is very unlikely that autophagy was induced all the time – rather autophagy would have been induced between periods of normal autophagy. Rapamycin is a drug designed for long-term use, and while it has some side-effects in patients and mice due to mTOR inhibition, these do not appear to be mediated by autophagy. While one may argue that the side-effect profile of rapamycin is outweighed by its potential benefits in many of these devastating diseases, it would be desirable to identify compounds that are better tolerated, since one may need to treat patients who are at risk for developing these diseases for decades. Hence we have tried to identify drugs that act independently of mTOR (Table 1).

**Chemical inducers of mTOR-independent autophagy**

The first hint that there may be mTOR-independent pathways controlling autophagy was the discovery that intracellular inositol 1,4,5-trisphosphate (IP$_3$) levels negatively regulate autophagy (41). We have shown that autophagy can be induced by lowering intracellular inositol or IP$_3$ levels, independently of mTOR. Lithium and other mood-stabilizing agents used for treatment of bipolar disorder, like carbamazepine and sodium valproate, enhanced the clearance of autophagy substrates by reducing intracellular inositol or IP$_3$ levels (41,42) (Fig. 3). The ability of lithium to induce autophagy is due to inhibition of inositol monophosphatase (IMPase), which prevents inositol recycling, leading to depletion of cellular inositol and inhibition of the phosphoinositol cycle (41,43). Accordingly, the specific IMPase inhibitor L-690 330 mimicked the effects of lithium on the clearance of autophagy substrates. Sodium valproate induces autophagy by inhibiting inositol synthesis and decreasing IP$_3$ levels (41,44). Consistent with a role of IP$_3$ on autophagy, pharmacological inhibition of IP$_3$ receptor (IP$_3$R) by xestospongin B also induces autophagy (45). It was further shown that xestospongin B induces autophagy by disrupting
the IP$_R$-beclin 1 complex, which can also be modulated by Bcl2 levels (46) (Fig. 3).

Lithium and sodium valproate reduced mutant huntingtin aggregation/toxicity in HD cell models and protected against neurodegeneration in Drosophila models of HD (41,47,48). Recently, lithium treatment in ALS patients and mouse models was found to increase survival and attenuate the disease progression (19). All the ALS patients on lithium treatment for 15 months survived, but approximately 30% of control patients receiving riluzole died (19). Apart from lithium's neuroprotective effects (49), this fascinating but preliminary result was attributed partly due to autophagy upregulation (19).

In order to identify novel pathways regulating autophagy that may be relevant to neurodegenerative diseases, we and others have performed chemical screens. Three major screens have been reported so far. The first of these screens started in the yeast, Saccharomyces cerevisiae, and aimed to identify small molecule chemical modifiers of the growth inhibitory effect of rapamycin (50). Of 50,729 compounds tested in a high-throughput screen, a number of small molecule inhibitors (SMIRs) and enhancers (SMERs) of the cytostatic effects of rapamycin were identified and subsequently analyzed in a secondary screen in mammalian cells by analyzing the clearance of A53T mutant α-synuclein (a good autophagy substrate) in the absence of rapamycin, as putative modulators of autophagy. We confirmed that SMERs 10, 18 and 28 were positive regulators of autophagy acting independently of rapamycin. These SMERs increased autophagosome synthesis and enhanced the clearance of model autophagy substrates, like A53T α-synuclein and mutant huntingtin fragments. Autophagy induced by SMERs was mTOR-independent. Furthermore, these SMERs were protective in a Drosophila model of HD. Further screening of the structural analogs of these 3 SMERs identified 18 additional small molecules that enhanced the clearance of aggregate–prone proteins (50).

In an attempt to identify novel potential therapeutic agents capable of inducing mTOR-independent autophagy, we screened a library of 253 compounds, comprising of FDA-approved drugs and pharmacologically active compounds belonging to different classes with known biological activities, by analyzing their effects on the clearance of known autophagy substrates, like the A30P and A53T α-synuclein mutants, in a stable inducible PC12 cell line. From the primary screen, we identified several mTOR-independent autophagy enhancers, such as L-type Ca$^{2+}$ channel antagonists (verapamil, loperamide, amidarone, nimodipine and nitrendipine), a K$^+$ ATP channel opener (minoxidil) and a Gi-signalling activator (clonidine), whose mechanisms of action were linked in a cyclical manner (48) (Fig. 3). L-type Ca$^{2+}$ channel antagonists, as well as the K$^+$ ATP channel opener, prevent the influx of Ca$^{2+}$ and decrease intracytosolic Ca$^{2+}$ levels, leading to inhibition of Ca$^{2+}$-dependent cysteine proteases called calpains and induction of autophagy. This is consistent with an earlier finding that raised intracytosolic Ca$^{2+}$ levels impair autophagy (51).

Pharmacological inhibition of calpain by calpastatin or calpeptin, or calpain knockdown, also induces autophagy. Conversely, calpain activation inhibits autophagy by cleaving and activating the α-subunit of heterotrimeric G-proteins (G$_{\alpha}$), which results in adenyl cyclase activation, and consequently, cAMP production (48,52). Reduction in cAMP levels by inhibitors of G$_{\alpha}$ (NF449) or adenyl cyclase (2'5'-dideoxyadenosine) induces autophagy. Likewise, imidazoline-1 receptor agonists (clonidine and rilmenidine) that also reduce cAMP levels, trigger autophagy (48). On the other hand, increased cAMP activates Epac, which in turn activates the small G-protein Rap2B, leading to activation of PLC-ε, and consequently increased IP$_3$ generation by PLC-ε-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP$_2$) (53). Consistent with our previous finding that generation of IP$_3$ inhibits autophagy, inhibition of the cAMP-Epac-Rap2B-PLC-ε-IP$_3$ pathway activates autophagy (48). Regulation of autophagy by intracellular IP$_3$ levels is most likely dependent on it being a signal for ER Ca$^{2+}$ release, as elevated cytosolic IP$_3$ levels bind the ER-resident IP$_3$R receptors to mobilise the ER Ca$^{2+}$ stores and increase cytosolic Ca$^{2+}$, which has autophagy-inhibitory effects (43,48). This creates an elaborate mTOR-independent autophagy pathway where Ca$^{2+}$-calpain-G$_{\alpha}$ signaling is linked to cAMP-Epac-PLC-ε-IP$_3$ in a potential cyclical fashion. Among the various autophagy enhancers identified in this screen that reduced huntingtin aggregation/toxicity in HD cell models, verapamil and clonidine were shown to protect against neurodegeneration in
HD fly models, and calpastatin, 2',5'-
dideoxyadenosine, verapamil and clonidine were protective in an HD zebrafish model (48).

Some of the L-type Ca\(^{2+}\) channel antagonists identified in our screen as autophagy inducers were also reported by an independent, high-throughput, image-based screen with 480 bioactive compounds, where the number of GFP-LC3 vesicles was measured as a readout (54). This screen yielded 8 compounds that trigger mTOR-independent autophagy and reduce expanded polyQ aggregates. These include fluspirilene, trifluoperazine (dopamine antagonist), pimozide, niguldipine, amiodarone, loperamide (Ca\(^{2+}\) channel blockers) and penitrem A (inhibitor of high conductance Ca\(^{2+}\)–activated K\(^{+}\) channels), which provide a number of potential therapeutic candidates for neurodegenerative disorders, as most of these compounds were FDA-approved drugs (54) (Fig. 3). Note that amiodarone identified both in this and our screens were classified as an mTOR-independent autophagy inducer (48,54). This contrasts with the recent screen by Roberge and colleagues, in which this compound was shown to inhibit mTORC1 at 10-50 fold higher concentration, compared to where it can induce autophagy without inhibiting mTOR (31,48,54).

The negative regulation of autophagy by intracytosolic Ca\(^{2+}\) levels was first suggested by Seglen and co-workers, consistent with the data from our recent screen (48,51) (Fig. 3). Autophagy was inhibited with agents that increase intracytosolic Ca\(^{2+}\) levels, such as thapsigargin (an ER Ca\(^{2+}/\)Mg\(^{2+}\) ATPase inhibitor that releases Ca\(^{2+}\) from ER stores), or ionomycin (Ca\(^{2+}\) ionophore that releases Ca\(^{2+}\) from intracellular stores) (51). Interestingly, thapsigargin blocks autophagic flux at two stages of the pathway. It increases LC3-II levels and vesicle numbers by impairing autophagosome-lysosome fusion and also reduces autophagosome synthesis by activating calpains (48). While another study reported an autophagy-inducing effect of thapsigargin, this only measured GFP-LC3 dots rather than autophagic flux, and our data suggested that these GFP-LC3 dots would increase due to impaired autophagosome-lysosome fusion – LC3 vesicle numbers can increase either if there is induction of autophagosome synthesis (increased autophagic flux) or inhibition of autophagosome-lysosome fusion (decreased flux) (55,56). Furthermore, calcium phosphate precipitates were shown to induce autophagy at early time-points, but blocked autophagosome-lysosome fusion after longer exposures (57,58). However, the compartments where these precipitates act in the context of autophagy are not known. Thus, these studies suggest complex roles for Ca\(^{2+}\) in autophagy.

Another chemical screen for identifying inhibitors of polyQ-mediated protein aggregation in vitro identified trehalose (a disaccharide) as an inhibitor of mutant huntingtin aggregation, which reduced toxicity in HD cell models and attenuated disease pathology in a mouse model of HD (59). This protective effect of trehalose was suggested to be mediated by its ability to act as a chemical chaperone through its binding to the polyQ-expanded mutant huntingtin and influencing its protein folding and aggregation. However, we have shown that trehalose enhances the autophagic pathway independently of mTOR, thereby increasing the clearance of mutant aggregate-prone proteins (60). Additionally, trehalose protected against pro-apoptotic insults via autophagy (60). The myriad of protective properties of trehalose acting as autophagy inducer and chemical chaperone, coupled with its lack of toxicity, may be of benefit in the treatment of neurodegenerative disorders.

Additive effects of mTOR-dependent and mTOR-independent autophagy pathways

The existence of mTOR-dependent and -independent pathways regulating autophagy allows the combined use of different perturbations in order to increase the autophagic clearance of aggregate-prone proteins. For instance, although lithium induces autophagy in an mTOR-independent manner by inhibiting IMPase, it also inhibits glycogen synthase kinase-3\(\beta\) (GSK-3\(\beta\)) that activates mTOR (47,61). This mTOR activation acts to partially inhibit the autophagy-inducing effects of lithium action via IMPase inhibition (47). We have shown that treatment with rapamycin impedes the GSK-3\(\beta\)-dependent activation of mTOR that occurs with the simultaneous treatment with lithium, thereby eliminating the undesirable effects on autophagy resulting from mTOR activation. Combinatorial treatment with rapamycin and lithium enables greater autophagic clearance of mutant huntingtin in HD cell models and exerts a greater protection against the neurodegeneration in HD fly models, compared to either treatment alone. Consistently, lithium treatment in an HD fly...
model with heterozygous TOR mutation rescued against neurodegeneration to a greater extent than the heterozygous TOR mutation alone (47). Moreover, this strategy may also benefit from the cytoprotective effects of GSK-3β inhibition occurring as a result of lithium treatment due to activation of the β-catenin/Tcf pathway, which may serve as an additional protective effect in the context of neurodegenerative diseases where there are secondary apoptotic insults (47,62).

We have further shown that simultaneous treatment with rapamycin and other mTOR-independent autophagy inducers, such as trehalose, calpains or the SMERs, result in a greater upregulation of autophagy than the single treatments alone (48,50,60). The combined therapy approach may minimize the side effects arising from these treatments by lowering the required doses of each compound, thereby providing a safer strategy for long-term treatments. Therefore, the use of combination treatment with lower doses not only provides additive mechanisms for enhancing autophagy, but also may abrogate undesirable effects resulting from the perturbations of these signalling pathways on their own.

Future prospects of autophagy as a therapeutic strategy

Recent advances in the field of autophagy have implicated its role in various physiological and pathological conditions, such as development, longevity, cancer, infectious diseases and cardiovascular diseases (10). Indeed, autophagy is a critical pathway that regulates the clearance of diverse intracellular pathogens (63). We have shown that two of the autophagy-inducing SMERs could reduce the number of viable intracellular mycobacteria in primary macrophages in an autophagy-dependent manner, providing evidence for their benefit in infectious diseases as well (64). Thus, chemical inducers of autophagy offer great potential for future studies and could also be utilised in various contexts outside neurodegeneration, either in the treatment of disease conditions where autophagy serves as a protective pathway, or in the investigation of signalling pathways regulating autophagy.

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References


**Figure legends**

**Figure 1. Autophagy as a protective pathway for neurodegenerative diseases.**  
Autophagy is a major degradation pathway for the clearance of various intra-cytosolic, aggregate-prone, toxic proteins associated with neurodegenerative diseases. Chemical induction of autophagy by autophagy enhancers trigger cellular signalling pathways leading to formation of double-membrane, cytoplasmic structures called phagophores. These structures elongate and engulf mutant aggregate-prone proteins along with portions of the cytoplasm to form autophagosomes. Autophagosomes then ultimately fuse with the lysosomes to form autolysosomes, where their contents are degraded by acidic lysosomal hydrolases. Enhancing autophagic clearance of these mutant aggregate-prone proteins results in reduction of mutant protein aggregates and toxicity, which is protective in several models of neurodegenerative diseases.

**Figure 2. Regulation of autophagy by mTOR pathway.**  
Autophagy is negatively regulated by mammalian target of rapamycin (mTOR), which is downstream in the mammalian target of rapamycin (mTOR) pathway, comprising cAMP-Epac-PLC-ε-IP3 and Ca2+-calpains-Gsα pathways, which has multiple drug targets for neurodegenerative diseases. Intracellular cAMP levels are increased by adenylyl cyclase (AC) activity, thus activating Epac, which then activates a small G-protein Rap2B, thereby activating phospholipase C-ε (PLC-ε). PLC-ε mediates the production of inositol 1,4,5-trisphosphate (IP3) from phosphatidylinositol 4,5-bisphosphate (PIP2), thereby increasing IP3 levels that bind to endoplasmic reticulum (ER)-resident IP3 receptors (IP3R), leading to release of Ca2+ from the ER stores. Intracytosolic Ca2+ levels are also increased by L-type Ca2+ channel agonists. Elevated intracytosolic Ca2+ activates calpains, which then cleave and activate Gsα. Activation of Gsα, in turn, increases adenylyl cyclase activity to elevate cAMP levels, thereby forming a loop. Activation of this pathway inhibits autophagy. Multiple drug targets acting at distinct stages in this pathway trigger autophagy, such as imidazoline-1 receptors (I1R) agonists (clonidine and rilmenidine) and adenylyl cyclase inhibitor [2′5′-dideoxyadenosine (2′5′ddA)] which decrease cAMP levels, agents lowering inositol and IP3 levels (carbamazepine and sodium valproate), inositol monophosphatase (IMPase) inhibitors that also reduce inositol and IP3 levels (lithium and L-690,330), Ca2+ channel blockers (verapamil, loperamide, amiodarone, nimodipine, nitrrendipine, nifedipine and pimozide), calpain inhibitors (calpastatin and calpeptin) and Gsα inhibitor (NF449). Furthermore, inhibition of IP3R by xestospongin B also induces autophagy by disrupting the IP3R-beclin 1 complex, and consequently the Beclin1-beclin 1 autophagy-inhibitory complex. Enhancing autophagy through this mTOR-independent pathway is protective in various models of Huntington’s disease.
Table 1

List of autophagy enhancers for neurodegenerative diseases and their mode of action

<table>
<thead>
<tr>
<th>Autophagy enhancers</th>
<th>Mode of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamycin, CCI-779, Glucose, Glucose-6-phosphate, Torin1, Perhexilene, Niclosamide, Rottlerin</td>
<td>Inhibit mTORC1</td>
<td>8, 20, 30, 31, 37, 39</td>
</tr>
<tr>
<td>Lithium, L-690,330</td>
<td>Inhibit IMPase and reduce inositol and IP₃ levels; mTOR-independent</td>
<td>41, 47</td>
</tr>
<tr>
<td>Carbamazepine, Sodium valproate</td>
<td>Reduce inositol and IP₃ levels; mTOR-independent</td>
<td>41, 48</td>
</tr>
<tr>
<td>Verapamil, Loperamide, Amiodarone, Nimodipine, Nitrendipine, Niguldipine, Pimozide</td>
<td>Ca²⁺ channel blocker; reduce intracytosolic Ca²⁺ levels; mTOR-independent</td>
<td>48, 54</td>
</tr>
<tr>
<td>Calpastatin, Calpeptin</td>
<td>Calpain inhibitor; mTOR-independent</td>
<td>48</td>
</tr>
<tr>
<td>Clonidine, Rilmenidine</td>
<td>Imidazoline-1 receptor agonist; reduce cAMP levels; mTOR-independent</td>
<td>48</td>
</tr>
<tr>
<td>2′,5′-dideoxyadenosine</td>
<td>Adenylyl cyclase inhibitor; reduces cAMP levels; mTOR-independent</td>
<td>48</td>
</tr>
<tr>
<td>NF449</td>
<td>Gs α inhibitor; mTOR-independent</td>
<td>48</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>K⁺,ATP channel opener; mTOR-independent</td>
<td>48</td>
</tr>
<tr>
<td>Penitrem A</td>
<td>Inhibits high conductance Ca²⁺-activated K⁺ channel; mTOR-Independent</td>
<td>54</td>
</tr>
<tr>
<td>Fluspirilene, Trifluoperazine</td>
<td>Dopamine antagonist; mTOR-independent</td>
<td>54</td>
</tr>
<tr>
<td>Trehalose</td>
<td>Unknown; mTOR-independent</td>
<td>60</td>
</tr>
<tr>
<td>SMER10, SMER18, SMER28, SMER analogues</td>
<td>Unknown; mTOR-independent</td>
<td>50</td>
</tr>
</tbody>
</table>

cAMP: cyclic AMP; IMPase: Inositol monophosphatase; IP₃: Inositol 1,4,5-trisphosphate; mTOR: Mammalian target of rapamycin; mTORC1: mTOR complex 1; SMER: small molecule enhancer of rapamycin
Figure 1

Induction by autophagy enhancers → Mutant aggregate-prone proteins → Lysosome → Autophagy → Autophagosome → Autolysosome → Neurodegeneration → Reduction of mutant protein aggregates and toxicity → Degradation of mutant aggregate-prone proteins
Figure 2

Rapamycin
FKBP12

mTORC1
Subunits: Raptor, mLST8, PRAS40

CCI-779, Glucose, Glucose-6-phosphate, Torin1, Perhexiline, Niclosamide, Rottlerin

Autophagy

ULK1
Atg13
FIP200
Chemical inducers of autophagy that enhance the clearance of mutant proteins in neurodegenerative diseases
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