Insulin-degrading enzyme: an ally against metabolic and neurodegenerative diseases

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SHORT RUNNING TITLE: Role of IDE in metabolic and neurodegenerative diseases

No conflicts of interest were declared.

ABSTRACT

Insulin-degrading enzyme (IDE) function goes far beyond its known proteolytic role as a regulator of insulin levels. IDE has a wide substrate promiscuity, degrading several proteins such as amyloid-β peptide, glucagon, islet amyloid polypeptide (IAPP) and insulin-like growth factors, that have diverse physiological and pathophysiological functions. Importantly, IDE plays other non-proteolytical functions such as a chaperone/dead-end chaperone, an E1-ubiquitin activating enzyme, and a proteasome modulator. It also responds as a heat shock protein, regulating cellular proteostasis. Notably, amyloidogenic proteins such as IAPP, amyloid-β and α-synuclein have been reported as substrates for IDE chaperone activity. This is of utmost importance as failure of IDE may result in increased protein aggregation, a key hallmark in the pathogenesis of beta cells in type 2 diabetes mellitus and of neurons in neurodegenerative diseases such as Alzheimer's and Parkinson's disease. In this review, we focus on the biochemical and biophysical properties of IDE and the regulation of its physiological functions. We further raise the hypothesis that IDE plays a central role in the pathological context of dysmetabolic and neurodegenerative diseases and discuss its potential as a therapeutic target.

Keywords: Insulin-degrading enzyme, Insulin, Amyloid-β, α-synuclein, Type 2 diabetes mellitus, Neurodegenerative disorders, Therapeutics

INTRODUCTION

Insulin-degrading enzyme (IDE) was first described in 1949 by Mirsky and Broh-Kahn, who reported its degradative activity over insulin [1]. Besides insulin, this protease catalyses several other substrates such as islet amyloid polypeptide (IAPP, also known as amylin) [2], glucagon [3], insulin-like growth factor (IGF)-I and -II [4], amyloid-β peptides (Aβ) [5], and others [6]. Moreover, IDE plays biological roles in protein quality control mechanisms such as chaperone function [7], E1 ubiquitin-activating enzyme [8] and as a heat shock response protein [9].

Considering the proteolytic action of IDE on insulin, its dysregulation has been widely investigated in the context of type 2 diabetes mellitus (T2DM) [10,11]. However, given its degradative capacity for amyloidogenic peptides such as Aβ, and its aggregation suppression activity over amyloidogenic proteins, it has also been investigated as an important regulator of Alzheimer's disease (AD) [12,13] and Parkinson's disease (PD) [14] pathogenesis. In this review, we aim to dissect the molecular mechanism(s) of IDE in physiological and pathological contexts in T2DM, AD and PD, and discuss if IDE is a novel druggable target for these pathologies.

Overview of IDE localisation, structure, and degradative function

The human *IDE* gene contains 25 exons [15], with six transcripts reported: four (3.5, 3.8, 5.5 and 9 kb) contain the catalytic site sequence, while the remaining two (2.0 and 4.0 kb) lack exon 3 that encodes the active site sequence. The four *IDE* transcripts containing the catalytic site sequence are found in tissues such as brain, liver, placenta, small intestine, colon, thymus, spleen, prostate, testis, ovary, heart, skeletal muscle, leukocytes, pancreas, kidney, peripheral nervous system, breast, and bone marrow [15,16]. Among these

transcripts, the 5.5 kb mRNA is the most abundant, corresponding to the 25-exon canonical form of IDE composed by the 3.5 kb *IDE* mRNA but with a longer 3' UTR [15].

IDE presents an "eclipsed distribution" widely spread in diverse subcellular localisations [17]. It is mainly cytosolic [18,19], but can also be found in the plasma membrane [18-20], multiple vesicular bodies (MVBs) [21-23], mitochondria [24] and peroxisome matrix (accounting for 10-20% of total IDE) [25]. IDE is also present in extracellular fluids such as cerebrospinal fluid (CSF) [22,26], wound fluid [27], blood plasma [28] and in erythrocytes [29,30]. Interestingly, IDE may be secreted from cells via exosomes, accounting for approximately 50% of IDE released to the media [21].

IDE is composed of four homologous αβ roll domains. Domains 1 and 2 comprise the N-terminal domains (IDE-N), while domains 3 and 4 are the C-terminal domains (IDE-C) [31]. These are connected via a 28-residue extended loop, leading to the formation of a triangular prism-shaped cavity whose surface comprises all domains with space to accommodate substrates such as insulin [31]. To become degradative, IDE requires conformational changes. In substrate-free conditions, IDE is present in a closed conformation [31,32] that cannot bind to substrates. Upon conformational rearrangements, IDE transits to an open state that allows substrate entry. However, proper catalysis only occurs if IDE returns to its closed state, followed by a reopening for release of proteolysis products. This "open-closed" conformational switch is essential for catalysis, as it allows for the correct positioning of residues important for either substrate recognition or degradation [31,32]. IDE naturally acquires a quaternary level of structure, existing in equilibrium between monomers, dimers, and tetramers [33,34]. Monomeric [34] and tetrameric IDE present lower activity than its dimeric form [33].

IDE can interact with and degrade a wide spectrum of substrates with little to no sequence homology. Various substrate features influence recognition and binding to IDE: i) substrate size, as peptides longer than 50-70 amino acids may not fit the catalytic chamber [31,35]; ii) substrate charge, since peptides with fewer positive charges like atrial natriuretic peptide, glucagon and IGF-II can more easily avoid the repulsive forces of IDE C-terminal, as opposed to the positively charged brain natriuretic peptide, glucagon-like peptide 1 and IGF-I that are poor substrates [31]; iii) substrate ability to undergo conformational changes and form β-sheets within IDE β-strands for anchoring in the catalytic site [31]; and iv) substrate N-terminal flexibility and ability to bind to IDE exosite [31,36].

Several factors further modulate IDE proteolytic ability, including IDE allosteric regulation by non-substrate molecules such as metal ions [37,38], ubiquitin [39], long-chain fatty acids [40], and ATP and other nucleotide triphosphates [41-43]. Different post-translational modifications such as Cys812 or Cys819 s-nitrosylation appear to be major negative regulators of IDE proteolytic activity. If combined with nitrosylation at Cys110, IDE proteolytic activity is totally eliminated [44]. In contrast, Cys178 nitrosylation has a protective role over IDE activity, even when Cys819 is nitrosylated [44]. IDE cysteine oxidation or alkylation also inhibits IDE, but to a lesser extent [44].

Multifunctionality of IDE: degradative-independent functions

The role of IDE in clearing insulin was first demonstrated upon *Ide* gene ablation in mice, which resulted in hyperinsulinaemia [45]. However, the specific deletion of *Ide* in liver does not induce hyperinsulinaemia [46], suggesting that, although the clearance of insulin is an important physiological function of IDE, it plays organ-specific functions. In fact, IDE is found in tissues that are not traditionally involved in insulin metabolism, such as testis and

brain [15]. IDE function is not exclusive for the degradation of insulin or other peptides. It is a moonlight enzyme that plays similar roles to heat shock proteins and chaperones, and modulates the Ubiquitin Proteasomal System (UPS) (Fig. 1) [47].

"Dead-end" chaperone:

IDE has been considered a key component of the proteostasis cellular machinery by proteolytically [19] and non-proteolytically [14] blocking aggregation of amyloidogenic peptides (Fig. 1A). Notably, IDE is enriched in tissues where amyloidogenic risk is elevated, such as pancreatic β-cells and the brain, which are continuously challenged with IAPP and Aβ aggregation, respectively [48]. Upon cellular protein accumulation, misfolded proteins tend to self-associate and form larger, stable and non-functional protein aggregates [49]. Although these aggregates are unlikely to fit the IDE degradative catalytic chamber, IDE interacts [14,50,51] and prevents their amyloidogenic process by acting as a molecular chaperone to facilitate their proper folding [48]. For example, IDE was reported to present chaperone-like activity over different amyloidogenic peptides related with neurodegenerative diseases, such as Aβ in the case of AD [7,52] and α-synuclein (aSyn) [14] that has a pivotal role in PD. However, the same dead-end chaperone activity has not been demonstrated for IAPP. As strong evidence of IDE chaperone function, a non-proteolytic variant of IDE (IDE-E111Q) can prevent the formation of Aβ aggregates [7].

Although Aβ is a degradable substrate of IDE [5], when proteolysis is disfavoured (i.e., suboptimal pH, oxidative and S-nitrosylative environment), IDE may trap Aβ monomers irreversibly within its cavity, acting as a "dead-end" chaperone and preventing the formation of toxic oligomers [53]. Specifically, the trapping of Aβ monomers occurs at the IDE exosite, which prevents amyloidogenic fibrillation of the $A\beta_{1-42}$ peptide in a non-proteolytic manner, rendering the aggregates less toxic and incompetent for seeding [7,53]. IDE can additionally

irreversibly bind to other peptides including ABri and Adan, forming stable complexes and thus also playing dead-end chaperone-like activity [53].

More recently, IDE was shown to similarly inhibit the amyloidogenic fibrillation of aSyn through a non-proteolytic mechanism [14,54]. IDE recognizes the C-terminal domain of aSyn and binds via the exosite [55], excluding aSyn from its degradative site [54]. Interestingly, aSyn binding was reported to increase IDE activity by stabilising the open conformation and facilitating access of other peptides to the catalytic chamber [54].

Heat shock protein:

In silico analyses suggest that the *IDE* gene promoter presents multiple heat shock elements, suggesting that IDE exerts a heat shock-like function [9]. For example, starvation, heat, or oxidative stress trigger *IDE* gene transcription, resembling the physiological expression pattern of other heat shock proteins [9] (Fig. 1B). IDE's role as a heat shock protein is further supported by its requirement for cell survival. In particular, downregulation of IDE in SH-SY5Y cells impairs their proliferation and viability and leads to a decrease of protein poly-ubiquitination, inhibiting the UPS system and triggering cellular apoptosis [56]. This further suggests that IDE may facilitate the identification of damaged proteins and physiologically regulate the activity of the UPS, which may trigger cell death upon failure [9].

Ubiquitin-proteasome pathway modulator and E1 ubiquitin-activating enzyme function:

IDE has been associated with control of the UPS by acting as an E1 ubiquitin activating enzyme and/or by directly modulating proteasome enzymatic activity [9] (Fig. 1C). The interaction between IDE and the proteasome was firstly determined in co-purification assays in several experimental models [57]. Recently, IDE was shown to interact with the

uncapped 20S and the single-capped 26S proteasome, but not with the double capped 30S proteasome nor the free 19S particle [58]. Binding of IDE to the 20S particle is believed to occur via a direct and high-affinity interaction with the subunits of the α-ring, important in regulating 20S gating mechanisms (opening and closing of the proteasome) [58]. IDE can regulate the proteasome system by dissociating the 26S and the 30S assemblies [58]. In addition, by competing with the 19S particle, it controls the equilibrium between the different proteasome components (30S, 26S and 20S) and consequently modulates proteasome substrate specificity [58]. Altogether, it is expected that IDE binding to the 20S proteasome induces conformational changes instead of a local effect, thus regulating proteasomal specificity and activity, working as a regulatory protein [58]. A higher expression of IDE is followed by dissociation of the regulatory protein 19S from the proteasome 20S, increasing the pool of uncapped 20S proteasome [59]. Although proteasomal abundance is variable between cells and tissues, the uncapped 20S particle is the common structure between the different types of proteasomes and is responsible for the ATP- and ubiquitin-independent proteolytic degradation of misfolded and oxidised proteins [9,47]. The increased levels of IDE together with the increased uncapped 20S proteasome pool suggests that the direct interaction between IDE and the 20S can modulate the activity and the composition of proteasome assemblies in response to cellular needs [58].

The role of IDE in controlling the UPS is further evidenced by its involvement in ubiquitination due to its ability to directly interact with ubiquitin [9]. So far, at least eight E1 enzymes are identified in humans [60]. Although E1s have different structures, they share a conserved domain that is very similar to MoeB and ThiF, bacterial proteins that control ubiquitin adenylation [61]. The IDE-C shares structural similarities with the MoeB-like domain of E1 enzymes and seems to present three of these ubiquitin binding sites, which are located far from its degradative site [8]. The ubiquitin-activating role of IDE in the

ubiquitination process was confirmed, since, in the absence of E1 enzyme but in the presence of E2 conjugating enzymes, it was able to promote the formation of ubiquitin dimers [8]. However, IDE does not play E2 enzymatic functions, because ubiquitin chain elongation reactions do not occur in the absence of an E2 [47]. Additionally, during the ubiquitin activation process, IDE is able to form adducts with ubiquitin with an ATP consumption rate similar to an E1 [8].

IDE DYSFUNCTION IN PATHOLOGY

Type 2 diabetes mellitus induced IDE alterations:

Diabetes is defined by the absence or limited production of insulin by the pancreas (type 1) and/or the ineffectiveness of insulin in target organs (T2DM). This insufficient function of insulin might translate into hyperinsulinaemia or be a consequence of hypersinsulinaemia resulting in hyperglycaemia [62-64]. Before the establishment of T2DM, individuals undergo an intermediate stage of the disease – prediabetes – characterised by impaired glucose tolerance and/or impaired fasting glucose [65].

Insulin levels are determined by the balance between the amount that is secreted and metabolised, the phenomenon known as insulin clearance. IDE has a central role in insulin clearance, whose impairment contributes to T2DM [62]. Research on *IDE* gene polymorphisms indicates that the rs1887922 and rs2149632 variants are associated with a higher risk of developing T2DM of about 26 and 33%, respectively [10]. Moreover, African American individuals, that have higher risk of developing T2DM, showed decreased hepatic IDE activity when compared to non-Hispanic whites [66]. Importantly, genetic evidence shows that the *IDE* gene controls insulin clearance in normoglycemic men but to a lesser extent in women [63], demonstrating an impact of gender on this process. Interestingly, the

diabetic and glucose intolerant rat model Goto-Kakizaki (GK) has two *Ide* mutations (H18R and A890V) [67]. Expression of these variants in a fibroblast cell line decreases insulin degradation (31%) compared to wild type IDE [67]. Furthermore, studies in *Ide* knockout (KO) mice identified an impairment in hepatic insulin clearance, followed by glucose intolerance and hyperinsulinaemia [45,68,69]. Despite glucose intolerance, decreased glucose-stimulated insulin secretion was observed in *Ide* KO mice after 13 weeks of high-fat diet [55] (Fig. 2). Additionally, we showed that *Ide* KO mice present testicular morphological changes and impaired sperm quality [70], recapitulating (pre)diabetes-associated infertility features (Fig. 2). Interestingly, liver-specific *Ide* KO mice also exhibit prediabetic features such as glucose intolerance and impaired insulin clearance [63]. Importantly, insulin clearance is a major regulator of insulin levels in the postprandial state, and *IDE* polymorphisms associate with postprandial insulin clearance in normoglycemic individuals but not in prediabetes or T2DM [63]. In fact, liver-specific *Ide* KO mice present no differences in C-peptide levels but display hyperinsulinaemia due to decreased insulin clearance [63] (Fig. 2). The regulatory effect of IDE on insulin clearance appears to be dependent of nitric oxide (NO), as it supresses IDE activity after a meal. In line with these observations, mice under high-fat diet showed impaired IDE activity and increased IDE S-nitrosylation [71] suggesting that this mechanism underlies impaired insulin degradation and leads to hyperinsulinaemia, insulin resistance and glucose intolerance. Indeed, hepatic *Ide* deletion decreased GLUT2, diminished glucokinase mRNA levels and decreased hepatic glucose uptake, leading to hyperglycaemia [63] (Fig. 2).

A further look at IDE loss-of-function led us to unveil its association with diabetes comorbidities, such as non-alcoholic fatty liver disease (NAFLD), characterised by suppressed insulin clearance [72,73]. Our studies show that liver-specific *Ide* ablation leads to hepatic triglyceride accumulation. Moreover, we found overexpression of CD36, a major

free fatty acid transporter, in liver and primary hepatocytes of the liver-specific *Ide* KO mice, explaining the observed increase in lipid droplets [63]. IDE was also found to have a role in skeletal muscle and white adipose tissue, where 7-month-old *Ide* KO mice show downregulation of insulin receptor in these insulin-sensitive tissues [63] (Fig. 2). Consequently, insulin stimulated glucose uptake in primary adipocytes was affected, thus contributing to hyperglycaemia.

Mice with streptozotocin-induced diabetes (T1DM) have lower IDE activity in adipose tissue, this reduction being reverted by insulin administration [74], which was also observed in *in vivo* and *ex vivo* experiments [75]. Using another approach, IDE inhibition led to the inhibition of insulin release at high glucose levels, or constitutive insulin secretion. Indeed, pancreatic β-cells *Ide* KO showed increased constitutive, but impaired glucose-stimulated insulin secretion [76] (Fig. 2), which might indicate a regulatory effect of IDE on this process. On the other hand, *in vitro* IDE silencing or pharmacological inhibition causes impaired glucose-stimulated insulin secretion [76]. T2DM patients also exhibit decreased pancreatic IDE levels [55]. Interestingly, both T2DM patients and β-cell *Ide* KO mice show increased pancreatic aSyn levels [55]. Curiously, patients with both NAFLD and T2DM have lower hepatic IDE levels than normoglycaemic NAFLD patients, or healthy controls [77], suggesting that decreased IDE levels have specific relevance in T2DM. The formation of islet amyloids is associated with IDE dysfunction. As IAPP is a known substrate of IDE whose amyloid aggregation promotes β-cell apoptosis, IDE degradative function plays a critical role in pancreatic physiology [2,50].

Due to its role in insulin clearance, IDE has progressively gained attention as a target for treating T2DM. IDE activation may decrease hyperinsulinaemia at earlier stages of T2DM, while it may be inhibited to increase insulin sensitivity at later stages where β-cell function has been compromised. Importantly, some drugs used to treat T2DM have effects

on IDE activity, in particular somatostatin and glitazones. However, the usage of direct inhibitors of IDE raises doubts due to its promiscuous nature (see Mechanisms of IDE based therapeutics section).

Impact of IDE in the brain:

Insulin metabolism has been studied mainly in insulin-sensitive organs, focusing on its role in regulating blood glucose levels. Interestingly, the presence of insulin receptors in the central nervous system (CNS) has been reported, suggesting that insulin might play a direct role in the brain [78]. The uptake of glucose in the brain is mainly insulin-independent, suggesting that insulin binding to neuronal cells may play an alternative role than glucose homeostasis [79]. Indeed, brain insulin signalling has been associated with multiple behavioural and metabolic effects, in particular in eating behaviour [80], peripheral metabolism [81] and cognitive functions (memory formation) [82].

Disturbances in brain insulin action are associated with ageing, dementia, AD and PD [83-85]. Interestingly, AD, PD and T2DM present systemic alterations driven by insulin metabolism dysfunction. Moreover, both AD and T2DM pathologies present impaired IDE activity in the brain [86]. Increased IDE S-nitrosylation and oxidation, described to impair its proteolytic activity, were found in the brains of AD patients compared to age-matched healthy brains [87]. Experiments in the GK rat model of diabetes or in *Ide* KO mice showed that IDE impairment/KO is associated with increased cerebral levels of Aβ [86,88], suggesting that the absence of IDE may trigger typical features of both AD and of T2DM. The consequent hyperinsulinaemia may produce negative feedback on IDE activity over other substrates, since insulin can compete with Aβ for IDE proteolytic activity, allowing Aβ accumulation and pathologic aggregation [5,12,26,89]. Due also to IDE failure, insulin metabolism dysregulation has been further proposed to lead to brain insulin resistance that

can mediate cognitive impairment and the development of AD [90,91]. Indeed, insulin signalling cascades regulate tau gene expression and phosphorylation [90,92], phenomena that are intrinsically associated with the molecular pathogenesis of AD.

Brain Ageing

Ageing is a major risk factor for T2DM and neurodegenerative diseases including AD and PD, and contributes to the progressive development of pathological features of these diseases. The failure of protein clearance mechanisms can result in the accumulation and aggregation of Aβ [93] and aSyn [94], which are typically found also in healthy aged individuals [95]. Although there are conflicting data regarding the alterations of IDE levels or activity [86], it is believed that IDE decreases with ageing, probably as a result of decreased production [96]. This age-associated IDE impairment may explain the accumulation of some of these amyloidogenic proteins either by being direct proteolytic substrates of IDE [97] or by being ubiquitination targets [8]. Amyloids are insoluble fibrous proteins enriched in β-sheet structures [97]. IDE is specific towards β-structure-forming substrates [31], which makes its proteolytic activity relevant against the formation of toxic oligomers associated with neurodegenerative diseases [48]. Additionally, epidemiological studies suggest that T2DM is associated with increased risk of developing ageing diseases such as AD (50%) [98] and PD (40%) [99,100]. Based on the previous premises, we can speculate that ageing-induced IDE failure could be a dysregulated mechanism underlying the relationship between T2DM and PD or AD.

Alzheimer's disease (AD)

AD is the most common neurodegenerative disorder, characterised by progressive decline of memory and cognitive functions, and behavioural changes such as aggression or agitation, anxiety, psychosis, and others [101,102]. In AD, the pathological accumulation of

Aβ extracellular neuritic plaques and of tau intracellular neurofibrillary tangles is associated with increased neuronal loss [103]. Various clearance systems are involved in the removal of soluble Aβ in the brain. Extracellularly, mechanisms such as Aβ efflux through the blood-brain barrier and secretion of peptidases contribute to the clearance of Aβ, while in the intracellular environment the main degradation pathways are the UPS and autophagy [104]. Among several cellular proteases able to degrade Aβ [105], IDE participates in both extracellular and intracellular Aβ degradation (Fig. 3 B-D) [106]. The involvement of IDE in this pathology was initially suggested by its role in maintaining the steady state levels of Aβ in the brain [68]. Further evidence supports this role, since IDE was shown to regulate the levels of extracellular Aβ and of the intracellular fragments of APP processing [107], thus preventing/suppressing further aggregation of Aβ [6] (Fig. 3 B, D).

The subcellular location of IDE is consistent with its role in the quality control system to eliminate aggregation-prone structures [12] that are found at endosomes and exosomes [21] (Fig. 3 B-D). The co-occurrence of IDE in these structures enables its interaction with newly formed peptides [12]. Moreover, the occurrence of IDE in exosomes might be associated with its transport to the extracellular space [21]. Based on this, IDE can degrade amyloidogenic peptides that are secreted to the extracellular milieu via exosomes, or that are already in the extracellular milieu [19]. Immunohistochemical studies of brain tissues derived from AD patients revealed the presence of IDE in intracellular neurofibrillary tangles as well as in extracellular senile plaques [108], supporting the hypothesis that IDE is an important defence against the accumulation of amyloidogenic proteins such as Aβ.

IDE is the major protease responsible for the clearance of Aβ [5], in particular of cytoplasmic monomers [106] in human hippocampus [109]. Alterations in the normal functioning of the hippocampus and cortex are respectively associated with early stages of cognitive (loss of memory) and non-cognitive impairment (motor impairment) in AD [110].

IDE proteolytic capacity and protein levels decrease during the earliest Braak stages of sporadic AD [106], possibly resulting in increased levels of toxic and oligomerised Aβ. Although there is controversy about the decrease of IDE levels in AD brains, a recent meta-analysis clarifies that AD patients exhibit decreased IDE levels in the cortex and hippocampus, while *IDE* mRNA levels and proteolytic activity remain unaltered [111]. We should note that decreased proteolytic capacity of IDE to degrade Aβ in AD was also reported [109]. Interestingly, regions with extensive Aβ deposition such as cortical microvessels from AD patients show higher levels of IDE (44%) but with reduced activity [112]. In AD transgenic mouse models, it is possible to detect co-deposition of IDE with Aβ plaques [113]. Curiously, after the formation of the first Aβ plaques in the cortex of APP-transgenic mice, the levels of IDE increase [113]. That IDE is detected at the periphery of Aβ fibrillar deposits [89], suggesting that increased IDE may represent a compensatory mechanism aimed to decrease Aβ levels and avoid its deposition.

The reduction of IDE's proteolytic activity in vulnerable regions of the CNS has been associated with its oxidation [114] and nitrosylation [87]. Interestingly, IDE is more oxidized in the hippocampus than in the cerebellum of both AD transgenic mice and in AD patients' brains [115]. This finding may represent the higher vulnerability of hippocampal neurons to AD pathology due to an increased oxidative environment [115]. IDE failure may result in an increase of Aβ levels and aggregation, which can further increase oxidative stress [114]. This vicious cycle may be responsible for AD onset and progression and allows the co-deposition of IDE with Aβ plaques. Interestingly, this co-deposition was found in vulnerable brain regions in AD but not healthy brains [116-118]. In agreement, higher levels of S-nitrosylated IDE were also reported in the brains of AD patients compared to age-matched healthy individuals [87].

Insulin signalling also plays an important role in AD. In particular, the activation of insulin receptor triggers a signalling cascade with multiple end targets including the inactivation of GSK3β [119,120]. If GSK3β is not properly inhibited, Aβ production and phosphorylation of the microtubule-associated protein tau increase [121,122], potentiating its aggregation into toxic intracellular neurofibrillary tangles. A fundamental process to maintain insulin receptor availability is its proper recycling [64,123]. After binding to insulin, its receptor triggers a signalling cascade and is internalised by endocytosis. IDE is also internalised in early endosomes and can clear insulin from the receptor, which is hypothesised to be important for these endosomes to follow the recycling pathway and to traffic insulin receptor back to the membrane. However, recycling may not occur if insulin fails to be degraded [124] and may cause insulin resistance by decreased availability of receptor at the membrane or slower insulin uptake, leading to hyperinsulinaemia. Defective insulin signalling will activate GSK3β [124], thereby increasing Aβ and tau pathology.

There is controversy about a genetic association between *IDE* and the development of AD. Both genetic linkage and allelic association in the *IDE* region of chromosome 10 have been reported in families with late-onset AD [88]. In fact, homozygous deletion of *Ide* in mice results in an early and marked elevation of cerebral Aβ [88]. Further studies showed that *IDE* polymorphisms such as rs3781239 and rs6583817 may influence the development of AD [125] and rs3781239 was implicated in AD susceptibility in a Chinese population [126]. Specifically, CC genotype carriers have a 4.89-fold higher risk for AD than CG and GG genotypes carriers [126]. rs6583817 polymorphism results in increased expression of IDE in cells and a decrease of Aβ levels in the plasma [127]. Given that IDE levels are reduced in affected regions of AD brain, mainly in the hippocampus [128], and that IDE overexpression in neurons decreases the brain levels of Aβ and inhibits the progression of

AD pathology in APP transgenic mice [129], it is plausible to hypothesise that IDE potentiation could reduce the risk of AD onset and development.

Parkinson's Disease (PD)

PD is the second most common neurodegenerative disorder [130], typically characterised by motor alterations such as resting tremor, rigidity, bradykinesia, gait impairment, postural instability and balance problems, and non-motor manifestations such as hyposmia, depression and cognitive issues [131]. A major hallmark of PD is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNPc), and the formation of proteinaceous aggregates - Lewy bodies (LBs) – primarily composed of aSyn [132]. aSyn is a peculiar long-lived protein since it has a natively unfolded structure that is highly prone to oligomerise, a process generally known as the amyloidogenic cascade. In as yet unclear pathological conditions, aSyn aggregation results in the formation of toxic oligomers up to insoluble fibres and aggregates that may be trapped in LBs or induce neuronal degeneration [133]. Of interest, we showed that glycation, a protein modification derived from the reaction between reducing sugars and amino groups of biomolecules, potentiates aSyn pathology by increasing its aggregation and cytotoxicity [134,135]. This modification is increased in hyperglycaemia conditions, suggesting that T2DM may influence the aggregation pattern of aSyn. T2DM patients have a higher risk of developing PD (40%), which is more evident (~380%) if T2DM occurs at younger ages (between 25-44 years) [99]. The relationship between both pathologies is further supported by the finding that aSyn levels are increased in the pancreatic β-cells of T2DM patients and IDE levels are decreased in the pancreas in these patients [55]. This accumulation of aSyn in the pancreas upon IDE loss was recapitulated in a mouse model (*Ide* KO) [55]. Notably, *IDE* locus polymorphisms associate with early onset PD [136]. Altogether, this evidence opens new questions and hypotheses regarding IDE function in PD.

The impact of IDE in PD is still unexplored. IDE cannot degrade aSyn mainly due to the size limitation of its proteolytic active chamber. However, the interaction between aSyn and IDE was already reported, in particular at IDE's exosite [14], suggesting that aSyn may modulate IDE function. The molecular mechanisms underlying the interaction of these proteins are supported by electrostatic attraction of the acidic and negatively charged C-terminal aSyn residues with the basic and positively charged exosite region in IDE-N [54]. This interaction can prevent the formation of aSyn oligomeric forms [14] (Fig. 3 K). Specifically, binding of IDE prevents aSyn amyloid formation by blocking the preferential site for self-interaction (aSyn C-terminal). In agreement, the decrease of IDE levels in the pancreas of T2DM individuals is followed not only by accumulation of aSyn, but also by its increased oligomerisation [55]. Additionally, interaction between aSyn and the IDE exosite potentiates IDE proteolytic activity over other small substrates [54]. Altogether, this evidence suggests that IDE impairment can result in accumulation and aggregation of aSyn, which is a major culprit in PD (Fig. 3 K). To our knowledge, it is not known if IDE is altered in the brain of PD patients, or if it has a protective role against aSyn pathogenesis in the brain, a research area we are currently investigating.

MECHANISMS OF IDE BASED THERAPEUTICS

Given the evidence for IDE dysfunction in several diseases, there is interest in targeting IDE as a therapeutic option. Several compounds that modulate IDE have been tested *in vitro* and/or *in vivo* (Table 1) and their modes of action are depicted in Figure 4. One attractive pathway to modulate IDE levels is the PI3K/AKT pathway, since PI3K is a main regulator of IDE expression [137]. This pathway can be activated either by direct regulation of PI3K [138] or by increasing the activity of upstream crosstalk mediators such

as extracellular signal-regulated kinases (ERK) [139], c-Jun N-terminal kinase (JNK) [140] and peroxisome proliferator-activated receptor γ (PPARγ) [141]. An important strategy for potentiating IDE levels may also be to increase of its secretion to the extracellular space [156, 157]. Moreover, IDE activation may also be achievable by ligands that either activate the previously mentioned pathways [158] or that induce positive cooperativity to increase catalysis [161, 162]. IDE may also be inhibited by modulating its active site, the exosite or other binding sites, or by inducing its oxidation or cysteine S-nitrosylation. Additionally, other viable strategies include modulating the IDE swing-door mechanism by locking the enzyme in a closed formation, which prevents protein degradation [142].

There is also increasing interest in allosteric drugs to modulate enzymes [143,144]. With its complex and unusual structure, IDE presents allosteric binding sites that can modulate its activity via, for example, ATP [41-43], anions [145,146], fatty acids [40,147] and ubiquitin [39]. Therefore, expanding our knowledge of the residues that participate in these interactions and conformational rearrangements may identify candidate residues as therapeutic targets. Other residues located at the interface of IDE domains or that shape the active pocket are equally relevant. It has been demonstrated that mutations in residues at domain interfaces, such as S137A, F364A and K898A, lead to an increased affinity for Aβ [148]. Additionally, the K898A mutation contributes to a higher catalytic rate of IDE, opposite to D426A that decreases IDE activity [148].

The development of drugs targeting IDE has been challenging due to their toxicity, potency, and selectivity. Moreover, it is difficult to modulate IDE proteolytic activity towards specific substrates or towards non-proteolytic substrates required for several physiological functions. This is even more relevant at a tissue-specific level, for example in increasing IDE brain levels but avoiding interference of IDE systemic degradative activity. This would be particularly important for AD and PD, to facilitate Aβ degradation or to prevent Aβ and aSyn

aggregation in the brain, without affecting IDE systemic regulation of insulin. To overcome these difficulties, it will be required to design and develop drugs that specifically target brain IDE, or even specific subtypes of brain cells, and that can have alternative modes of administration that allow crossing the blood-brain barrier. In this regard, one may take advantage of gene therapy via vanguard technologies such as nanoparticles, adenoassociated virus (AAV) or even exosomes. These could provide the means to modulate IDE levels via its overexpression or silencing, and the opportunity to express IDE with stabilising or destabilising mutations in allosteric sites that affect substrate turnover, or in residues that favour proteolytic or non-proteolytic functions of IDE. In addition, these strategies may facilitate the delivery of IDE to specific tissues or cells, enabling precision modulation of IDE at a cellular and molecular level.

To our knowledge, currently available compounds were developed only to modulate IDE proteolytic function. Since IDE is a multifunctional protein, it is also vital to develop compounds that may modulate IDE chaperone-like, heat shock protein and UPS functions, which could have pharmacologic potential for proteinopathies such as AD and PD.

CONCLUSION AND FUTURE PERSPECTIVES

IDE is typically known for its degradative ability to clear insulin. However, IDE is a central player in several cellular mechanisms [47]. It regulates the levels of other substrates such as Aβ [68], acts as a chaperone/dead-end chaperone of aggregation-prone proteins [7], modulates the UPS by interaction with the proteasome [58,149] or by its E1-activating enzyme function [8], and even acts as a heat shock protein [9]. However, it is unclear how these functions are coordinated. It is most likely that conformational rearrangements, the

intracellular environment, subcellular localisation, and tissue localisation affect all IDE functions [47].

Considering the pleotropic roles of IDE in cellular homeostasis, it is plausible to assume that its dysregulation has important consequences for the organism. These consequences may be at a systemic level, resulting in hyperinsulinaemia, insulin resistance and glucose intolerance [45] but may also be organ-specific. For example, patients with NAFLD specifically present decreased levels of IDE in the liver [77], while T2DM patients show decreased levels and activity of IDE in the pancreas, dysregulating insulin secretion and resulting in aSyn accumulation [55]. IDE failure in the brain also results in the accumulation of Aβ, a key phenotypic feature of AD [86,88].

IDE has a preference for interaction with proteins with amyloidogenic properties [48]. This is of particular importance in the brain, where proteinopathies such as AD and PD are dependent on the pathogenicity of key aggregation-prone proteins that contribute to neurodegeneration. Importantly, the cellular environment of these pathologies shares similarities with T2DM, including increased oxidative and nitrosative stress, impaired mitochondrial functions, and sustained inflammation [150]. With these associations, it is easy to understand why T2DM is an important risk factor for the development of both these neuropathologies [150-152]. Hence, it is probable that IDE dysfunction is one of the underlying links between T2DM and neurodegenerative diseases.

Although the exact catalytic mechanism of IDE is not yet fully clarified, there is knowledge of the biochemical and biophysical properties that contribute to the swing-door mechanism and its cooperativity [31,32]. Therefore, it is vital to design and develop chemical modulators that modulate IDE structure and its proteolytic features. It is also important to identify compounds that modulate IDE non-degradative functions such as chaperone, UPS,

and heat shock response in a tissue specific manner. This would be important as a putative therapeutic target for AD and PD, particularly for the case of PD where aSyn is not degraded by IDE, but its oligomerisation is suppressed by IDE chaperone activity [14]. Although IDE is a promising potential therapeutic target, it is also problematic and potentially hazardous as, for example, an increase of IDE would be beneficial to clear Aβ, but could result in hypoinsulinemia, dysregulating glucose homeostasis. Therefore, combinations of different routes of administration, together with compounds that modulate different IDE activities should be explored in the future.

Aside from the physiological impact of IDE and its therapeutic potential, it would be important to determine if IDE dysregulation identifies individuals at higher risk for metabolic or neurodegenerative diseases. These studies would improve the diagnostic potential of IDE and stratify individuals that would benefit from IDE compensation therapy.

Chronic diseases are a major concern in modern societies. The lack of efficient disease-modifying treatments is a crucial point in clinical practice that deserves thorough research and development. Due to its pleotropic functions, IDE represents a suitable candidate for managing several pathogenic events. Therefore, we envision IDE as a strong ally against metabolic and neurodegenerative diseases.

ACKNOWLEDGEMENTS

This work was supported by iNOVA4Health – UID/Multi/04462/2013, a program financially supported by Fundação para a Ciência e Tecnologia (FCT) / Ministério da Educação e Ciência, through Portuguese national funds and co-funded by FEDER under the PT2020 Partnership Agreement. It received further support from FCT: PTDC/BIM-MET/2115/2014 (MPM), PTDC/MEC-MET/29314/2017 (MPM) and from Sociedade Portuguesa de

Diabetologia: SPD, Portugal (MPM and HVM). MPM was supported by European Commission Marie Skłodowska-Curie Actions H2020 (grant agreements no. 734719). LS was supported by FCT fellowship SFRH/BD/144743/2019.

AUTHORS CONTRIBUTION

HVM and MPM conceived the idea and together with LS and MG designed the review. LS, MG and HVM performed the systematic review. LS, MG, MPM and HVM drafted the first version of the manuscript. All authors provided critical feedback, contributed to the final manuscript, and accepted the final version.

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TABLES

Table 1 - List of compounds that modulate IDE levels and/or activity.

Nr	Compound	Effect on IDE	Mechanism of action	Reference
1	Memantine	Upregulated expression	ERK pathway	[153,154]
2	Neuropep-1			[155,156]
3	Isoproterenol		JNK pathway	[139]
4	Norepinefrine			
5	Propanolol			[140]
6	4,5-dicaffeyolquinic			[157]
7	Pioglitazone		PPARγ pathway	[141]
8	Rosiglitazone			[158]
9 + 10	Ginsenoside Rg1 and Rg3			[158-160]
11	GW742			[161]
12	Leptin		PI3K pathway	[138]
13	ACP-105 + AC-186			[162]
14	British precursor protein-2 (BRI2)		Secretion	[163]
15	Somastatin	Upregulated expression and activator	Secretion and positive cooperativity	[164]
16	Polyunsaturated fatty acids		Positive cooperativity	[147,165]
17	IL-34		Microglia neuroprotective response	[166]
18	Troglitazone	Activator	PPARγ pathway	[167]
19	Retinoic acid receptor alfa agonists		GSK3β pathway	[168]
20	Apomorfine			[169,170]
21	NMDA	Downregulated expression	ERK pathway	[153]
22	Sevoflurane		Gene expression	[171]
23	GW9662		PPARγ pathway	[160]
24	Satured fatty acids	Inhibitor	Negative cooperativity	[40]
25	Peptide hydroxamic acid inhibitors		Locked close conformation	[142]
26	Benzoisothazalone-based compunds		Cysteine S-nitrosylation	[172]
27	BDM4476		Locked closed conformation	[173]
28	6bK		Catalytic pocket binding	[174]
29	Stabilized β-hairpin peptide B35		Direct interaction	[175]
30	Bacitracin			[176]
31	Cyclic dodecapeptide P12-3A			[177]

FIGURES

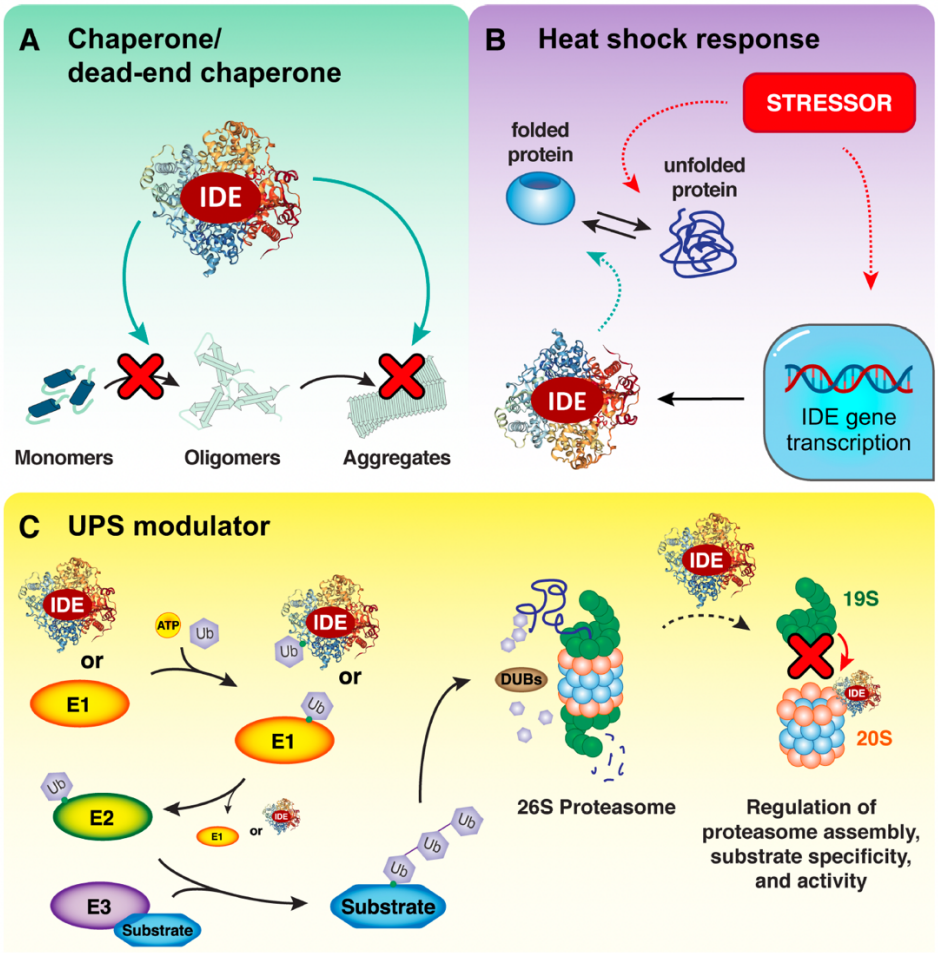

Figure 1. Alternative functions of IDE.

IDE is traditionally known for its degradative capacity. However, IDE is a "moonlight" enzyme with several other functions. **(A)** IDE acts as a chaperone or as a 'dead-end' chaperone preventing peptide/protein aggregation. **(B)** Upon stress, IDE expression increases in a heat shock protein like manner, for example acting in the unfolded protein response. **(C)** IDE can modulate the ubiquitin proteasome system (UPS) by playing ubiquitin-activating E1 enzyme activity. It can also compete with the 19S proteasome subunit for interaction with the 20S proteasome subunit, therefore dissociating the 26S and the 30S assemblies and altering proteasome substrate specificity. The activity of the proteasome is modulated by IDE depending on its intracellular concentration.

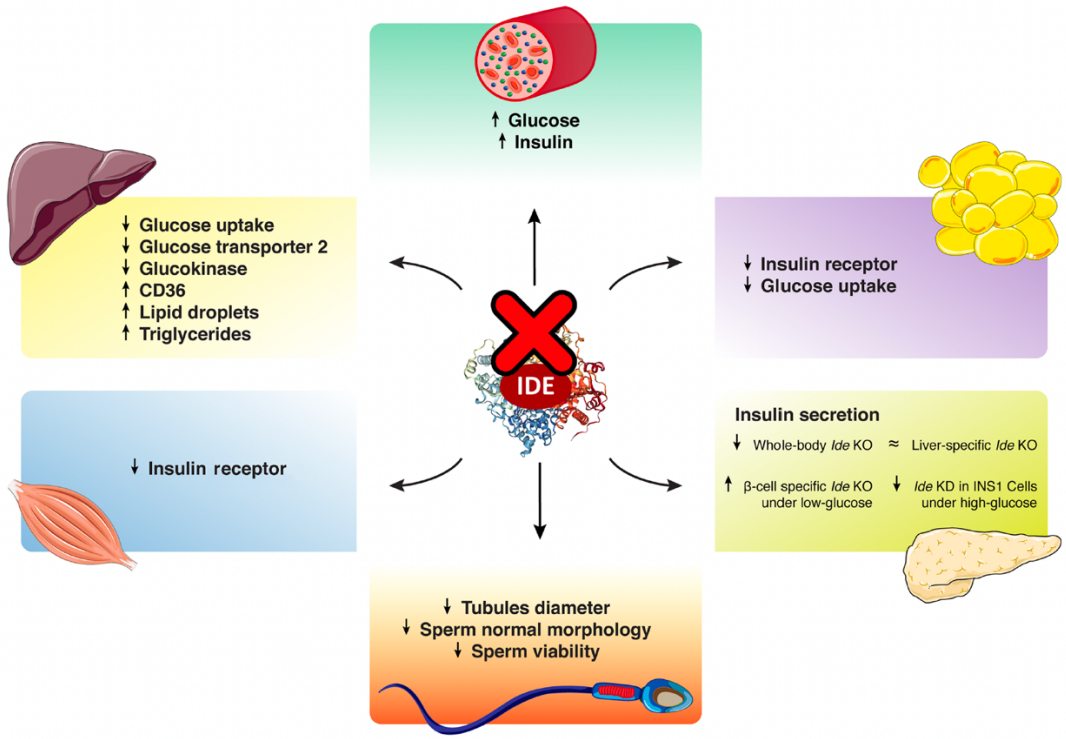

Figure 2. Systemic and organ-specific consequences of IDE deletion in the context of diabetes.

Whole-body deletion of IDE leads to hyperglycaemia and hyperinsulinaemia, hallmarks of T2DM [69], and results in insulin receptor downregulation in skeletal muscle and white adipose tissue [45], and male reproductive function impairment by decreasing seminiferous tubule diameter, sperm viability and normal sperm morphology [70]. Liver-specific *Ide* deletion leads to decreased glucose uptake and GLUT 2 levels, as well as decreased glucokinase mRNA levels [63]. Although CD36 levels decrease, triglyceride levels increase, resulting in more lipid droplets [63]. Regarding insulin secretion: whole-body *Ide* KO causes decreased glucose-stimulated insulin secretion [55], while liver-specific *Ide* KO has no differences in circulating C-peptide levels [63]; *in vivo* beta cell-specific *Ide* deletion leads to increased glucose-stimulated insulin secretion and circulating C-peptide levels with low glucose levels, but *in vitro Ide* silencing in INS1 cells cultured in high-glucose levels leads to impaired glucose-stimulated insulin secretion [76].

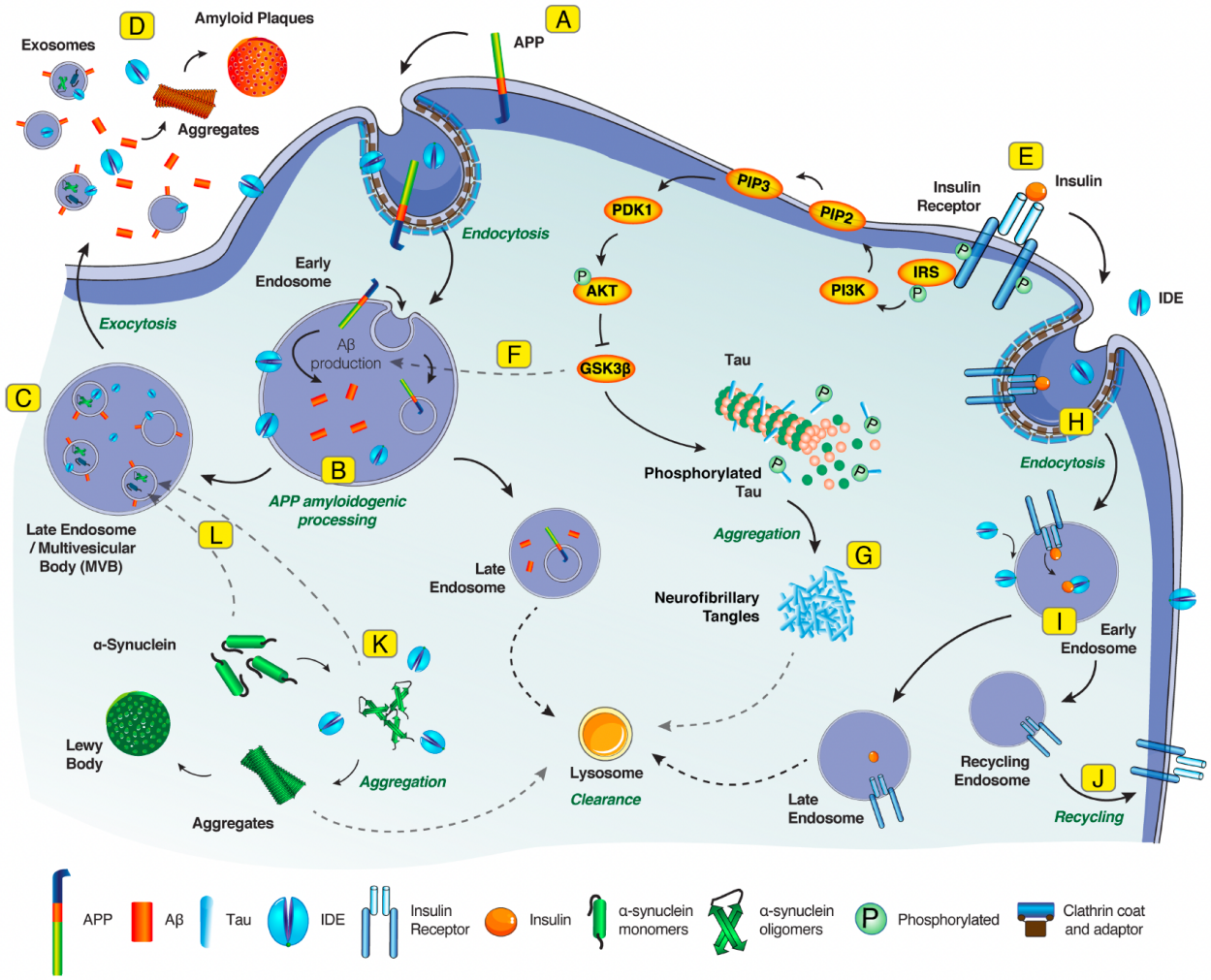

Figure 3. Crosstalk between IDE and Alzheimer's or Parkinson's disease pathogenic pathways.

(A) APP protein at neuronal plasma membrane [178] and extracellular or membrane-bound IDE [64] are internalised mainly via clathrin-mediated endocytosis. **(B)** APP amyloidogenic processing occurs at early endosomes by BACE1 and γ-secretases, forming Aβ peptides [179]. IDE is reported at the lumen or membrane of early endosomes and may degrade Aβ or prevents its aggregation in these structures. **(C)** Throughout endosome maturation, the early endosomal membrane invaginates forming inner luminal vesicles giving rise to multivesicular bodies, which fuse with the plasma membrane and secrete cargo via exosomes or other exocytosis process, releasing amyloidogenic Aβ. IDE may process Aβ or prevent its aggregation in exosomes [21]. **(D)** Extracellular Aβ may aggregate. However,

extracellular IDE or membrane-bound IDE (facing the extracellular space) can degrade Aβ or prevent its aggregation. IDE impairment could result in higher accumulation of Aβ at early endosomes, exosomes, and extracellular space, increasing its pathologic aggregation. **(E)** Insulin binding to the insulin receptor triggers a signalling cascade that increase IDE levels and inhibit the activity of GSK3β. This active protein **(F)** increases the production of Aβ and **(G)** induces tau phosphorylation. Tau hyperphosphorylation induces its aggregation and leads to the formation of neurotoxic neurofibrillary tangles, a hallmark of AD. **(H)** Upon insulin binding, the insulin receptor is internalized via endocytosis. **(I)** In early endosomes, the insulin molecule may require removal for the receptor to be inactivated and **(J)** recycled back to the plasma membrane. Failure in IDE-mediated insulin receptor recycling may decrease insulin signalling **(E)**, contributing to increased Aβ production. Insulin signalling failure also decreases IDE, which represents a vicious cycle with great impact in AD and PD pathogenesis. **(K)** Under pathological conditions, aSyn enters an amyloidogenic cascade, forming highly toxic oligomers and aggregates. IDE can suppress aSyn oligomerisation and toxicity. **(L)** aSyn species are reported in exosomes, where IDE may suppress aSyn aggregation and toxicity.

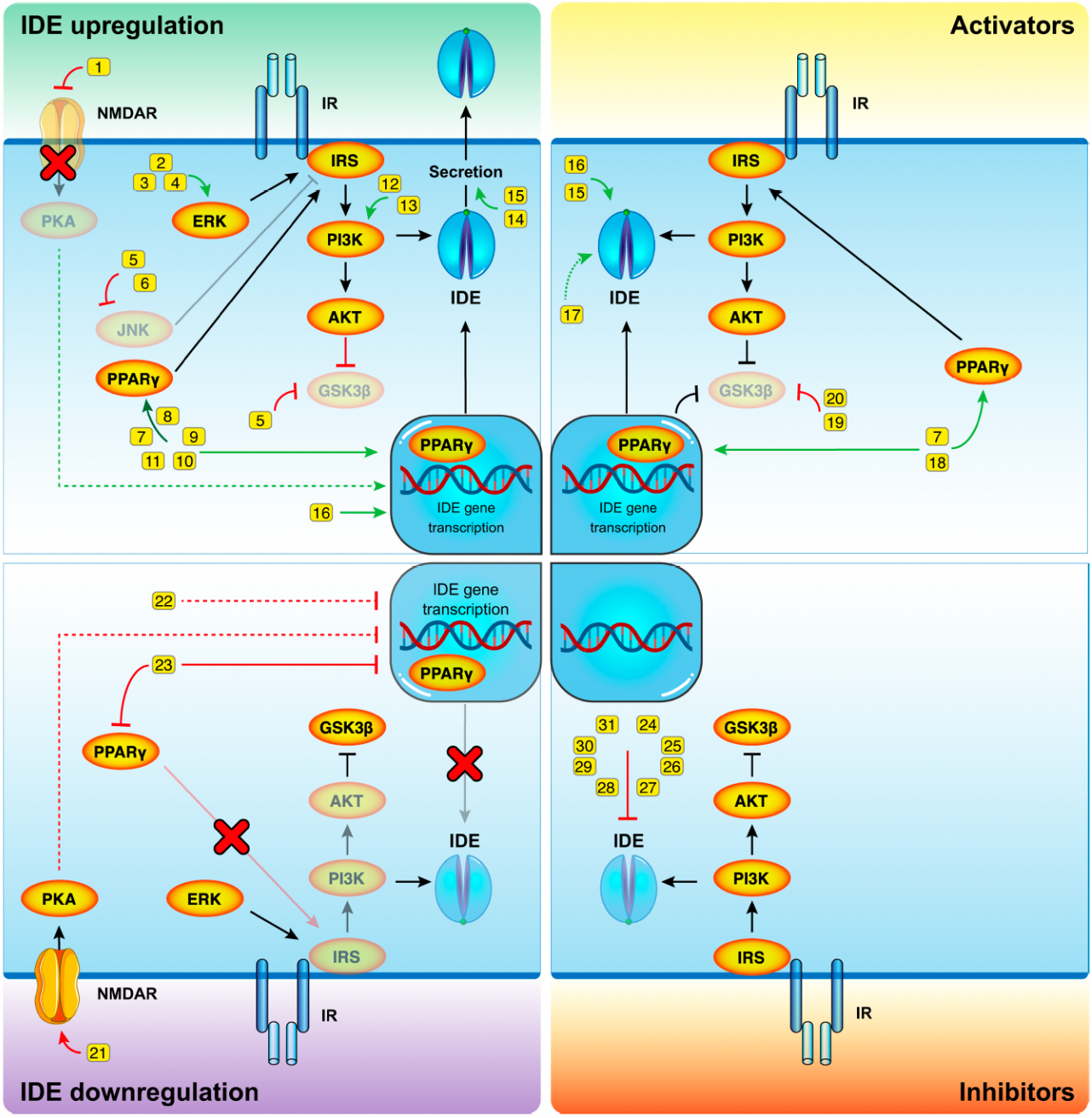

Figure 4 – Modulatory mechanisms of IDE.

Schematics of IDE levels and proteolytic activity modulation. Several compounds demonstrated to modulate IDE at a transcriptional level mainly via PI3K, PPARγ or PKA pathways, increasing IDE levels. Extracellular IDE can be increased by potentiating its secretion. IDE activity can be directly regulated via specific interactions with IDE. Compounds number (yellow) agree with Table 1.