Mechanisms of Ageing and Development 177 (2019) 46–54

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

# Protein aggregates and proteostasis in aging: Amylin and β-cell function

Michaela Press[a,b], Tobias Jung[a,c], Jeannette König[a], Tilman Grune[a,b,c,d,e], Annika Höhn[a,b,*]

[a] Department of Molecular Toxicology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), 14558 Nuthetal, Germany
[b] German Center for Diabetes Research (DZD), 85764 Muenchen-Neuherberg, Germany
[c] German Center for Cardiovascular Research (DZHK), 10117 Berlin, Germany
[d] NutriAct – Competence Cluster Nutrition Research Berlin-Potsdam, 14558 Nuthetal, Germany
[e] Institute of Nutrition, University of Potsdam, 14558 Nuthetal, Germany

ARTICLE INFO

*Keywords:*
Aging
Proteostasis
Protein aggregates
Human amylin
Proteasome
Autophagy

ABSTRACT

The ubiquitin-proteasomal-system (UPS) and the autophagy-lysosomal-system (ALS) are both highly susceptible for disturbances leading to the accumulation of cellular damage. A decline of protein degradation during aging results in the formation of oxidatively damaged and aggregated proteins finally resulting in failure of cellular functionality. Besides protein aggregation in response to oxidative damage, amyloids are a different type of protein aggregates able to distract proteostasis and interfere with cellular functionality. Amyloids are clearly linked to the pathogenesis of age-related degenerative diseases such as Alzheimer's disease. Human amylin is one of the peptides forming fibrils in β-sheet conformation finally leading to amyloid formation. In contrast to rodent amylin, human amylin is prone to form amyloidogenic aggregates, proposed to play a role in the pathogenesis of Type 2 Diabetes by impairing β-cell functionality. Since aggregates such as lipofuscin and β-amyloid are known to impair proteostasis, it is likely to assume similar effects for human amylin. In this review, we focus on the effects of IAPP on UPS and ALS and their role in amylin degradation, since both systems play a crucial role in maintaining proteome balance thereby influencing, at least in part, cellular fate and aging.

## 1. Introduction

Aging is a complex process involving multiple components affecting each other. Many theories exist trying to explain the intricate process. As a natural and universal process aging *per se* is not defined as a disease. However, aging is accompanied by the accumulation of cellular damage potentially resulting in age-related diseases influenced by genes as well as lifestyle and environmental factors. Studies in nematodes, fruit flies and mice revealed that genes involved in cellular stress response significantly affect aging (Dues et al., 2016; Hayflick, 2000; Lopez-Otin et al., 2013; Tosato et al., 2007; Zhang et al., 2015; Johnson et al., 2000; Guarente and Kenyon, 2000). Free radicals as well as non-radical reactive oxygen or nitrogen species (ROS and RNS), generated endogenously as a by-product of normal metabolism or exogenously due to environmental factors, contribute at least in part to the initiation of cellular stress. These species react with and damage cellular macromolecules, such as proteins, lipids and DNA. Oxidized or modified macromolecules accumulate and, in case of lipids and proteins, aggregate during aging due to reduced stress response able to affect further macromolecules (Grune et al., 2001; Levine and Stadtman, 2001; Sies, 1997; Ames et al., 1993; Halliwell and Chirico, 1993; Oliver et al., 1987; Davies, 1995). Misfolded and aggregated proteins are linked to several degenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease or even cancer (Grimm et al., 2011).

Lipofuscin is one of the predominant aggregates, whose abundance correlates positively with increasing age (Couve et al., 2012; Kumar et al., 2008; Mukherjee et al., 2009; Perse et al., 2013; Schmucker and Sachs, 2002). It is composed of oxidized proteins and lipids as well as carbohydrates and small amounts of trace elements (Benavides et al., 2002; Harman, 1989; Jolly et al., 2002). Several studies revealed interfering effects of lipofuscin with the main cellular degradation mechanisms by influencing proteasomal and also lysosomal activity as

*Abbreviations:* Aβ, β-amyloid; AD, Alzheimer's disease; ALS, autophagy-lysosomal-system; Atg, autophagy-related protein; ATP, adenosin-triphosphate; CGRP, calcitonin-gene related peptide; CMA, chaperone-mediated autophagy; DUB, deubiquitinating enzyme; EGFP, enhanced green fluorescent protein; ER, endoplasmic reticulum; IAPP, islet amyloid polypeptide; hIAPP, human islet amyloid polypeptide; Hsp, heat-shock protein; LAMP2A, lysosomal associated membrane protein 2A; LC3, microtubule-associated protein light chain 3; MODC, mouse ornithine decarboxylase; mTOR, mechanistic target of rapamycin; PA28, protein activator 28; PE, phosphatidylethanolamine; rIAPP, rodent islet amyloid polypeptide; RNS, reactive nitrogen species; ROS, reactive oxygen species; SQSTM1, sequestosome 1; T2D, type 2 diabetes; UCH-L1, ubiquitin carboxy-terminal hydrolase L1; Ub, ubiquitin; UPS, ubiquitin-proteasome-system

* Corresponding author at: German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany.
*E-mail addresses:* michaela.press@dife.de (M. Press), tobias.jung@dife.de (T. Jung), jeannette.könig@dife.de (J. König), tilman.grune@dife.de (T. Grune), annika.hoehn@dife.de (A. Höhn).

https://doi.org/10.1016/j.mad.2018.03.010
Received 27 February 2018; Accepted 22 March 2018
Available online 23 March 2018

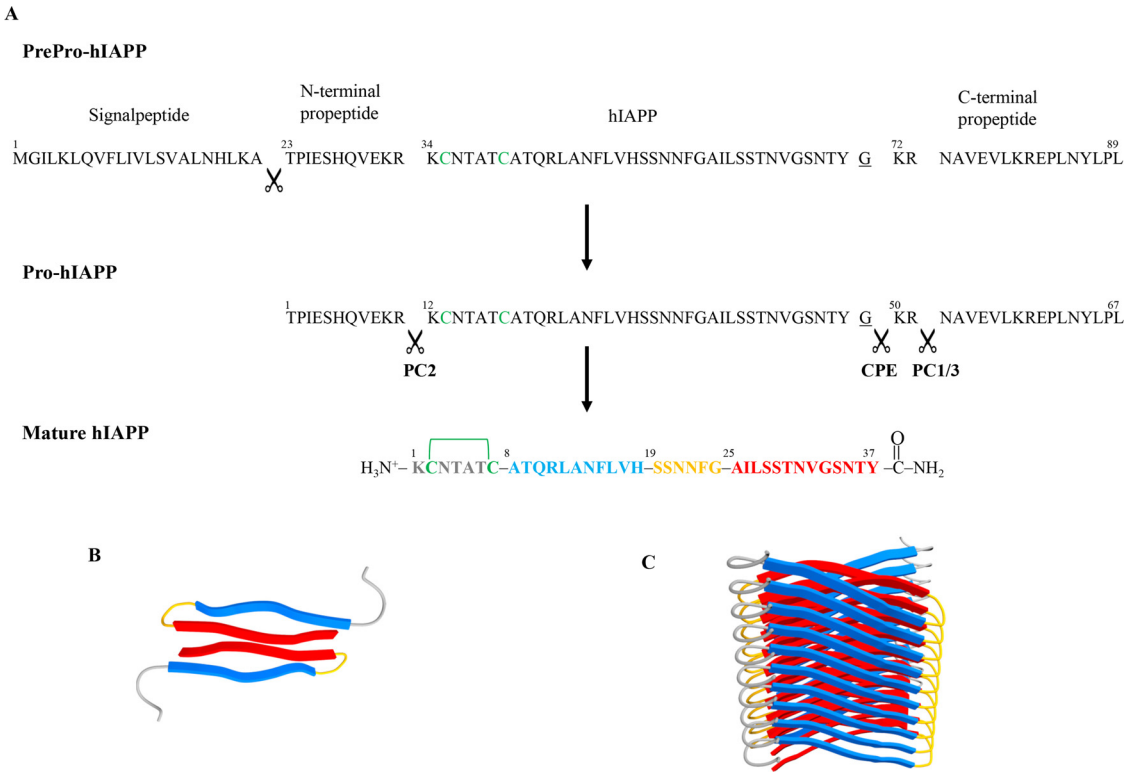


**Fig. 1.** Amino acid sequences and processing steps leading to mature hIAPP (A). Model of two U-shaped hIAPP monomers arranged to each other (B) and of a hIAPP fibril (C).
(A) The 89-amino acid PrePro-hIAPP is cleaved to the 67-amino acid Pro-hIAPP, which is consequently cleaved to the mature hIAPP by prohoromone convertase 2 and prohormone convertase 1/3. Carboxypeptidase E-processing takes place at the C-terminal glycine (underlined), which is finally amidated. The mature hIAPP consists of 37 amino acids and a disulfide-bond between cysteine 2 and 7 (green colored, for other colors see Fig. 1B). (B) Human IAPP is shaped in U-form. Amino acid residues 1–7 are outside of the U-shape. Residues 8–18 build the N-terminal β-sheet (blue colored), residues 25–37 the C-terminal β-sheet (red colored) and residues 18–22 form the loop between the two sheets (yellow colored). U-shaped hIAPP arrange in an in-registered order, in which the C-terminal sheets are located side by side. (C) U-shaped in-registered organized hIAPP monomers arrange on top of each other to form columns, building up a fibril. Each column consists of C-terminal and N-terminal β-sheets. To further form amorphous aggregates, fibrils randomly bind to each other (not shown). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

well as generation of ROS and induction of apoptosis (Couve and Schmachtenberg, 2011; Höhn et al., 2011, 2010; Powell et al., 2005; Sitte et al., 2000a; Suter et al., 2000). The occurrence of lipofuscin is connected to pathologies such as macular degeneration and Alzheimer's disease (Wolf, 2003; Giaccone et al., 2011).

Besides oxidatively damaged protein aggregates, amyloids occur as a different type of aggregated proteins. Amyloidogenic proteins show β-sheet conformation, tightly linking these proteins to fibrillogenesis, but also amorphous aggregates can be formed (Tjernberg et al., 1999; Dobson, 1999). Currently, about 50 different proteins are determined forming such amyloidogenic structures, among them β-amyloid (Aβ) and human amylin (Marshall et al., 2014; Westermark, 2005). Until now, it is not clear, whether amyloid accumulation increases with age, although one human study showed an age-dependent increase in amylin-derived deposition in pancreatic islets (Su et al., 2012). Amylin secretion is also found to be altered in aged mice and plasma concentrations of amylin were shown to be increased in aged obese mice compared to young ones (Takada et al., 1996; Leckstrom et al., 1999). In addition, amyloids are clearly linked to age-related diseases. The accumulation of Aβ is a well-known hallmark of AD and, furthermore, human amylin was shown to accumulate in Type 2 diabetes (T2D) patients. Human amylin is known to have detrimental effects on pancreatic β-cells resulting in an impairment of β-cell functionality and finally in cell death (Westermark, 2005; Mukherjee et al., 2015; Hoppener et al., 2000; Hull et al., 2004).

Since the ubiquitin-proteasomal-system (UPS) and the autophagy-lysosomal-system (ALS), as the main cellular proteolytic systems, serve in maintaining proteostasis, both systems strongly influence cellular fate and consequently the aging process (Chondrogianni et al., 2015; Madeo et al., 2015). Decreased activity of cellular turnover and proteostatic mechanisms are, amongst others, typical characteristics of aging leading to an accumulation and aggregation of non-degraded proteins. Studies revealed a decline in the amount of proteasomes in aging cells as well as in proteasomal activity in most tissues during aging possibly due to altered expression or modification of UPS enzymes or proteasomal subunits (Grune et al., 2001; Levine and Stadtman, 2001; Couve et al., 2012; Perse et al., 2013; Smith et al., 1991; Bulteau et al., 2002; Sitte et al., 2000b,c; Kapphahn et al., 2007; Tsakiri et al., 2013; Chondrogianni et al., 2003; Ferrington et al., 2005; Bulteau et al., 2000; Okada et al., 1999). Also autophagy seems to be in part impaired in age as a result of altered expression of ALS relevant proteins (reviewed in Höhn et al., 2017). As mentioned before also age-related highly cross-linked protein aggregates such as lipofuscin interfere with UPS and ALS. The proteasome recognizes lipofuscin as a substrate trying to degrade this material. However, lipofuscin is non-degradable by the proteasome and therefore also inhibits this protease by preventing the degradation of other oxidized and non-functional proteins (Höhn et al., 2011). Lipofuscin is also found to accumulate in lysosomes and thereby decreases lysosomal enzyme activity (Terman and Brunk, 2004; Höhn and Grune, 2013; Höhn et al., 2012). Impaired proteolytic mechanisms – as a long-term consequence – lead to disturbed proteostasis and accumulation of non-degradable protein aggregates resulting in a cellular malfunction and cell death.

Studies also revealed altered UPS and ALS in AD. In AD patients and

transgenic mice, impaired proteasomal ubiquitinating/deubiquitinating enzymes and alterations in proteasomal subunits as well as a defective autophagic flux are found. Because both systems play important roles in Aβ generation and clearance, dysfunctional UPS and ALS lead to an accumulation of aggregated Aβ (reviewed in Zhang et al., 2017).

Since protein aggregates such as lipofuscin and Aβ have highly interfering effects on UPS and ALS, it is likely to assume that human amylin as a peptide susceptible to form amyloid aggregates affects proteolytic systems in the β-cell. This may lead to impaired β-cell functionality due to an imbalance in the proteome, which could contribute at least in part to the pathogenesis of T2D. Here, we review the known effects of human amylin on the mechanisms of protein degradation pathways and amylin's clearance by ALS/UPS in pancreatic β-cells.

## 2. Properties of amylin

Amylin, also called islet amyloid polypeptide (IAPP), is a 37-amino acid residue peptide, which is mainly co-expressed and co-secreted with insulin in a ratio between 1:10 and 1:100 (IAPP:insulin) in β-cells, but also in some other cell types (Betsholtz et al., 1989; Kahn et al., 1990; Mulder et al., 1995, 1994; Toshimori et al., 1990; Westermark et al., 1987; German et al., 1992; Kahn et al., 1991; Mulder et al., 1996). Initially, IAPP is expressed as 89-amino acid PrePro-peptide, which is subsequently cleaved to the Pro-peptide by peptidases in the endoplasmic reticulum (ER) and finally to the mature IAPP by prohormone convertase 2, prohormone convertase 1/3 and carboxypeptidase E in the Golgi apparatus. Additionally, the amidation of the C-terminal glycine-residue is required for the full biological activity of IAPP (Marzban et al., 2004; Prigge et al., 1997; Sanke et al., 1988; Steiner et al., 1993; Wang et al., 2001). Fig. 1A shows the amino acid sequences of human PrePro-IAPP, Pro-IAPP and mature IAPP with its cleaving and modification sites.

The amino acid sequences of IAPP and calcitonin gene-related peptide (CGRP) have strong similarities. Hence, IAPP belongs to the calcitonin peptide family. So far, there is no evidence for an amylin-specific receptor. However, some studies indicate the occurrence of calcitonin receptors heterodimerized with receptor activity-modifying proteins (RAMPs), which have a high affinity for IAPP in the brain of mice and rats (Barth et al., 2004; Christopoulos et al., 1999; McLatchie et al., 1998; Muff et al., 1999; Tilakaratne et al., 2000; Ueda et al., 2001; Westermark et al., 1986).

IAPP has multiple physiological roles and acts in an endocrine as well as auto- and paracrine manner affecting glucose metabolism and energy balance. The physiological function has not been completely elucidated; current knowledge is summarized in Fig. 2.

Blood glucose levels are not only controlled by the uptake of glucose from blood into peripheral tissues such as skeletal muscle, but also by the entry of glucose from digested carbohydrates into the blood stream. It is well-known that insulin is responsible for facilitating the transport from the blood stream into insulin-dependent tissues (reviewed in Carnagarin et al., 2015). Amylin is partially responsible for controlling the other part, the uptake of glucose into the blood stream by slowing down gastric emptying (Clementi et al., 1996; Young et al., 1996). Therefore, IAPP may mediate centrally by direct action in the brain in a receptor-dependent way (Ueda et al., 2001; Wickbom et al., 2008). However, IAPP may also mediate suppression of gastric emptying directly in the gut, since binding sites for IAPP are found in the stomach fundus of rats (Bhogal et al., 1992) and mRNA expression of IAPP is detected in the gastrointestinal tract (Ferrier et al., 1989; Nicholl et al., 1992; Miyazato et al., 1991). Additionally, one study also reveals relaxation effects of IAPP on rat ileal smooth muscles (Mulder et al., 1997).

Regarding regulation of energy homeostasis, amylin controls food intake and body weight by acting as a satiation signal mediated directly in the central nervous system (Rushing et al., 2000a) (reviewed in Lutz, 2005). It is also effective as an adiposity signal to control energy expenditure (Wielinga et al., 2007,2010). Some studies revealed that amylin is able to interact with and enhance the effects of other hormones in this context, for instance peptide YY (Roth et al., 2007), insulin (Rushing et al., 2000b) and leptin (Roth et al., 2008). Especially leptin shows a much higher impact on suppression of food intake and body weight in combination with amylin administration (Trevaskis et al., 2010a,b; Turek et al., 2010). The impact of amylin on insulin and glucagon release is contrary. Some studies discovered that IAPP seems to inhibit insulin secretion (Wang et al., 1993; Degano et al., 1993; Kogire et al., 1991), whereas others were not able to show this effect (Pettersson and Ahren, 1990; Broderick et al., 1991; O'Brien et al., 1990). Regarding glucagon, some publications reveal a, at least in part, suppressed secretion from islet α-cells leading to a decreased conversion of glycogen to glucose (Gedulin et al., 1997; Young, 2005; Akesson et al., 2003; Panagiotidis et al., 1992).

Amylin seems to have additional endocrine effects in the organism. It is assumed to influence bone metabolism due to the sequence similarity to CGRP by inhibiting osteoclast activity and stimulating osteoblast proliferation and activity (Zaidi et al., 1990; Villa et al., 1997). Furthermore, IAPP apparently plays a role in renin-angiotensin-system and has vasodilatory effects (Wookey et al., 1998; Brain et al., 1990). However, the physiological roles of amylin are still not fully understood and should be further investigated.

IAPP of most mammalian species, including humans (hIAPP), is prone to form fibrils in β-sheet conformation resulting in conversion to the above mentioned amyloids. In contrast, rodent IAPP (rIAPP) does not form such amyloidogenic structures due to the presence of proline-residues in certain positions of the amino-acid-sequence (Johnson et al., 1992; Jaikaran et al., 2001; Westermark et al., 1990). Infrared spectroscopy, electron microscopy and solid-state nuclear magnetic resonance studies revealed the following structural model of human amylin fibrils: Every fibril is composed of columns, which are built up by U-shaped IAPP monomers packed on top of each other. Due to the U-shaping, two parallel β-sheets are formed within every column, one N-terminal (residue 8–17) and one C-terminal (residue 25–37) β-sheet. Residues 18–22 form a loop between both β-sheets and a disulfide bond exists between cystein 2 and 7. Hydrogen bonds stabilize the monomers. Fig. 1B shows a model of U-shaped so called "in-registered" organized hIAPP-monomers, what means an arrangement of the C-terminal sheets next to each other in an anti-parallel way, and Fig. 1C of a hIAPP fibril (Middleton et al., 2012; Luca et al., 2007; Wiltzius et al., 2008).

hIAPP-aggregates accumulate mainly extracellularly. Nevertheless, in β-cells small amounts were also found intracellularly (Jaikaran and Clark, 2001; Paulsson et al., 2006). By now, several hypotheses of the cytotoxic mechanisms of human amylin on β-cells have been published, but none of the described cellular processes is fully understood. The cytotoxic effects range from membrane disruption to ROS generation, induction of apoptosis, impact on inflammasome, ALS and UPS and suggested mechanisms *via* ER stress. In the last years, research increasingly focused on IAPP oligomers, since they are assumed to be more toxic than amorphous aggregates (Engel et al., 2008, 2006; Janson et al., 1999; Mattson and Goodman, 1995; Schubert et al., 1995; Zhang et al., 2008, 2002, 1999; Masters et al., 2010; Casas et al., 2007; Rivera et al., 2011; Mirzabekov et al., 1996; Anguiano et al., 2002). Fig. 3 summarizes the most important hypothesized cytotoxic effects of hIAPP on β-cells.

## 3. Amylin and ubiquitin-proteasome-system

The UPS is besides the ALS one of the main proteolytic systems in mammalian cells. Both are involved in preservation of proteostasis and constantly adjust the proteome to maintain a functional set of proteins and thus cellular functionality. The UPS recognizes and degrades (oxidatively) damaged and dysfunctional proteins, therefore partially

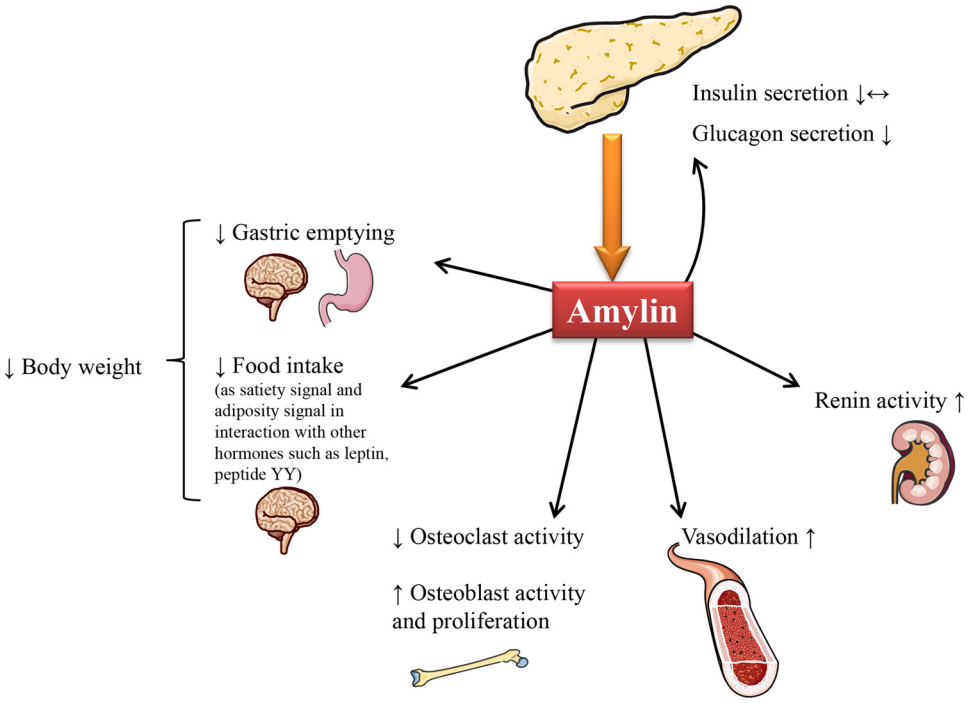


**Fig. 2.** Physiological role of amylin.
IAPP has endocrine as well as auto-/paracrine effects. It is involved in slowing down gastric emptying and reducing food intake, by acting in the brain and probably also in the stomach, and consequently reduces body weight. For that, amylin also works synergistically with other hormones such as leptin. Due to its similarity to the calcitonin-gene related peptide, it affects also bone metabolism by decreasing osteoclast activity. Additionally, amylin influences vasodilation and the renin-angiotensin-system. Amylin's autocrine effects on pancreatic β-cells are controversial. It seems to reduce glucagon release. However, insulin secretion was found to be decreased as well as unaltered.

preventing the accumulation and aggregation of damaged proteins. Fully functional proteins, which are no longer needed or pass through a normal turnover, are also removed by the UPS. The proteasome is the most important cytosolic protease in mammalian cells, removing more than 90% of the oxidatively damaged proteins (Jung et al., 2009, 2006).

Studies investigating the mutual effect of IAPP and UPS are rare and the results are incoherent. In 2007, Casas et al. performed the first investigations regarding IAPP and UPS. They used the murine pancreatic insulinoma β-cell line Min6, a frequently investigated β-cell line due to their characteristics similar to isolated islets, and primary cultures of human pancreatic islets. Treatment of Min6 cells with hIAPP showed a significant decrease of proteasomal activity compared to rIAPP treatment. The measurement of 26S proteasomal activity was performed with cells transiently expressing ZsGreen-MODC fluorescent protein and the detection of the intracellular amount of ubiquitinated proteins in transiently UbG76V-EGFP expressing cells. Similar results were shown in a fluorometric 20S proteasome assay with extracts from human pancreatic islets after treatment with hIAPP (Casas et al., 2007).

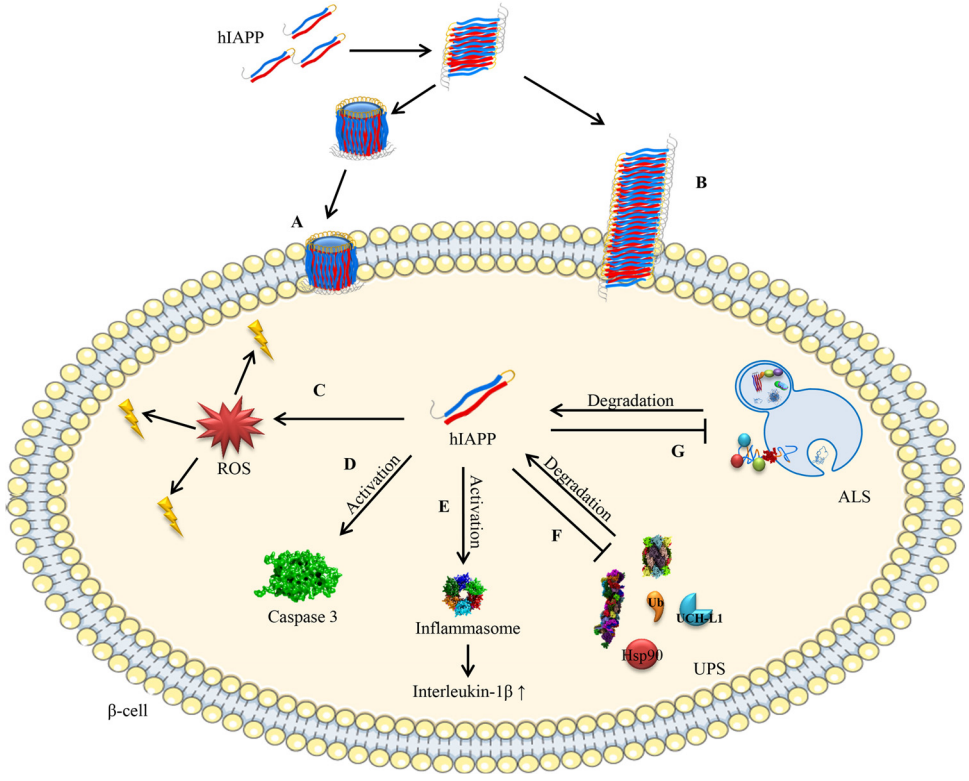


**Fig. 3.** Possible toxic effects of human IAPP on β-cells.
hIAPP monomers form oligomers and might build channel-like structures, which penetrate the cell membrane leading to an increased permeability for ions (A). hIAPP oligomers puncture the cell membrane, where they proceed development to long thin fibrils resulting cell membrane rupture (B). hIAPP induces the formation of ROS able to damage cellular macromolecules such as proteins, lipids and the DNA (C). hIAPP initiates the cleavage of procaspase 3 to caspase 3 leading to apoptosis *via* pathways involving c-jun, Fas and p53 (D). hIAPP activates the inflammasome resulting in interleukin-1β mediated inflammation (E). The UPS (F) and ALS (G) are both involved in degradation of hIAPP and *vice versa* hIAPP has interfering effects on the UPS **(F)** and the ALS **(G).**

The 20S proteasome is the core particle consisting of multiple subunits forming four rings stacked on top of each other. The outer alpha rings are in charge of substrate recognizing and regulating the access of the substrates into the proteasomal chamber, where the inner beta rings provide their proteolytic activity. To form the 26S proteasome, so called 19S regulator subunits are necessary. Binding of one regulator to an alpha ring of the 20S proteasome leads to the formation of the 26S proteasome, whereas binding of two 19S regulator domains forms the 30S proteasome, in the literature often also termed as 26S proteasome. The regulator subunits modulate proteasomal activity and substrate specificity in an ATP-dependent way. Energy released by hydrolysis of ATP is needed for the unfolding of proteins, but not for the proteolytic degradation itself. Hence, the 26S and 30S are able to degrade natively folded and fully functional proteins, in contrast to the unbound 20S (Jung et al., 2009; Arendt and Hochstrasser, 1997; Bedford et al., 2010; Groll et al., 2000; Orlowski and Wilk, 2000; Rabl et al., 2008; Wehmer and Sakata, 2016).

Besides the 19S regulator, specific tags binding to the substrates are required for degradation of fully functional and natively folded proteins by 26S proteasome. Several ubiquitin molecules, a ubiquitously occurring protein comprised of 76 amino acids, form a short "polyubiquitin" chain, linked to the substrate. This system of highly specific substrate polyubiquitination and deubiquitination controlling proteasomal degradation is strongly regulated by specific ubiquitination enzymes called E1–E3 and deubiquitination enzymes (DUBs), such as Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) (Clague et al., 2015; Tai and Schuman, 2008; Ristic et al., 2014; Bishop et al., 2016).

In contrast to the investigation of Casas et al. other studies showed controversial findings regarding proteasomal activity. Transduction of isolated human islets with adenoviruses expressing hIAPP did not show any differences in 20S proteasomal activity compared to rIAPP transduced cells, but both resulted in an increase of proteasomal activity compared to non-transduced control cells. Furthermore, no differences in proteasomal activity could be observed in islets of hIAPP transgenic rats and wild-type rats (Costes et al., 2011). In another study, treatment of RIN-m5F, a rat pancreatic insulinoma β-cell line, and isolated human pancreatic islets with hIAPP or rIAPP revealed inhibitory effects of both forms on proteasomal activity (Singh et al., 2016).

Furthermore, microarray analysis reported a downregulation of cytosolic Heat-shock proteins (Hsp) 90AA1, 90AB1 and ER-related Hsp90B1 after hIAPP-treatment (Casas et al., 2007). Hsp90 is a chaperone assisting in protein degradation by the 26S proteasome and preventing 20S proteasome inactivation due to oxidative stress. Downregulation of these chaperones could also contribute to the decline of proteasomal activity (Imai et al., 2003; Conconi et al., 1998).

Impairment of the 26S proteasome normally leads to accumulation of ubiquitinated proteins. Indeed, an increased amount of ubiquitinated proteins in hIAPP-treated Min6 cells was found. Also hIAPP transgenic rats and hIAPP transduced INS 832/13, a rat insulinoma cell line, showed an accumulation of polyubiquitinated proteins in comparison to wildtype rats and rIAPP transduced cells (Casas et al., 2007; Costes et al., 2011), whereas hIAPP treatment of RIN-m5F cells did not result in an elevation of polyubiquitinated proteins (Singh et al., 2016). An accumulation of ubiquitinated proteins may also be a hint for a deficiency of other UPS components, such as DUBs. UCH-L1, one of the DUBs, is an important enzyme in the UPS controlling the proteasomal entry of ubiquitinated proteins by deubiquitinating and thereby preventing substrate degradation (Ristic et al., 2014; Bishop et al., 2016). Individuals with type 2 diabetes show diminished levels of UCH-L1 in combination with a decreased proteasomal activity and consequently an accumulation of polyubiquitinated proteins (Costes et al., 2011). hIAPP transgenic rats showed decreased mRNA levels of UCH-L1 and a marked decline in UCH-L1 protein levels. Furthermore, hIAPP or rIAPP transduced INS 832/13 cells confirmed downregulation of UCH-L1 as a specific event for amyloidogenic hIAPP, but not for non-amyloidogenic rIAPP. Chemical inhibition as well as knockdown of UCH-L1 in INS 832/13 led to increased apoptosis, measured by cleavage of caspase-3 and its substrate Poly(ADP-ribose) polymerase (PARP). Furthermore, elevated ER stress, shown by raised levels of CCAAT-enhancer-binding protein homologous protein (CHOP) and diminished levels of eukaryotic initiation factor 2 (eIF2) were detected. Both hIAPP and rIAPP transduction in addition to UCH-L1 knockdown further increased apoptosis compared to scramble-transfected cells, hIAPP to a greater extent than rIAPP. This indicated that non-amyloidogenic rIAPP is also harmful under certain conditions possibly due to the higher protein burden by IAPP transduction in the cells (Costes et al., 2011).

Another important question besides the effects of hIAPP on the UPS is, whether the proteasome is responsible for the degradation of IAPP. Therefore, studies with proteasome activity modulators were performed. Inhibition of the proteasome with lactacystin and epoxomicin resulted in an increase of hIAPP content and toxicity in hIAPP-treated Min6 and RIN-m5F cells. Toxicity was measured by analysis of apoptosis (caspase-3 cleavage) and by viability tests. Conversely, stimulation of the proteasome with protein activator 28 (PA28) was reducing the cytotoxic effects of hIAPP. Furthermore, inhibition of the proteasome also resulted in an elevated amount of endogenously expressed rIAPP. This led to the assumption that the proteasome plays at least in part a role in degradation of both uptaken hIAPP and also endogenous non-amyloidogenic rIAPP (Casas et al., 2007; Singh et al., 2016).

To confirm the role of the proteasome in hIAPP degradation, immunoprecipitation studies were performed. Interactions between hIAPP and the α4 subunit of the 20S proteasome were found mainly in the nucleus of RIN-m5F cells and of human islets. The question arises, whether IAPP is degraded by 20S proteasome or also by 26S proteasome in an ubiquitin-dependent way. *In vitro*-studies revealed that ubiquitination of IAPP is not necessarily required for the interaction of hIAPP with 20S proteasome. In contrast, immunoconfocal microscopic studies reported a strong colocalization of hIAPP and ubiquitin in hIAPP treated cells and human islets. Internally expressed hIAPP was also shown to be ubiquitinated. Additionally, rpn8, an important regulatory subunit of the 19S complex, co-precipitated with hIAPP and α4. hIAPP further colocalized with Hsp70 and Hsp90. These results indicated that an ubiquitin-dependent interaction cannot be excluded. Furthermore, an additional *in vitro* study showed proteasomal degradation of hIAPP in both aggregation-inhibiting and aggregation-prone conditions leading to the assumption that aggregation of hIAPP is not required for its degradation by the proteasome (Singh et al., 2016; Rivera et al., 2014).

In summary, the UPS seems to play a role in the turnover of both hIAPP and rIAPP. Dysfunctional proteasomal degradation mechanisms, as they occur during aging or due to covalently cross-linked protein aggregates, could therefore lead to the accumulation of ubiquitinated proteins and amyloidogenic aggregates such as hIAPP, exacerbating the toxic cellular effects of human amylin. Human amylin apparently impairs the UPS, more precisely proteasomal activity and the expression of cytosolic chaperone Hsp90 and deubiquitination enzyme UCH-L1.

## 4. Amylin and autophagic-lysosomal-system

The second important cellular proteolytic system besides the UPS is the ALS. A major characteristic is the dynamic membrane reorganization resulting in the uptake and degradation of substrates. The ALS is able to incorporate protein aggregates, macromolecules, also from the extracellular space, and even whole cell organelles, while the UPS is only capable to degrade proteins. It also plays an important role in a wide array of cellular processes, such as differentiation, growth control, inflammation, immune system, energy metabolism (Mizushima and Levine, 2010; Wang and Levine, 2010; Qian et al., 2017; Settembre et al., 2013; Dowling and Macian, 2018; Dengjel et al., 2005).

Currently three different types of autophagy, macroautophagy, microautophagy and chaperone-mediated autophagy, which differ in the pathways and mechanisms of substrate delivery to lysosomes, are

described. During macroautophagy, cytosolic substrates are incorporated by a sequestering organelle – the phagophore or isolation membrane –, which develops to the autophagosome by expanding and sealing. Completing autophagosome formation is followed by fusion with lysosomes, content release and degradation by lysosomal enzymes. Macroautophagy is in comparison to the other autophagy types highly inducible and is regulated by a variety of signaling pathways, which enable appropriate response to different environmental conditions. Atg proteins (autophagy related proteins) are involved in these mechanisms by forming complexes leading to recruitment and induction of downstream Atg proteins. During microautophagy the lysosomes invaginates cytosolic material resulting in vesicle budding into the lysosomal lumen and its subsequent degradation. The third type is chaperone-mediated autophagy (CMA). Chaperones such as Hsc70 (Heat shock cognate 70) bind to substrates containing the amino acid sequence KFERQ and transfer them to the lysosome leading to the translocation of the substrate into the lysosomes mediated by co-chaperones, such as Hsp90, and lysosome-associated membrane protein type 2A (LAMP2A). For a more detailed description of autophagic mechanisms see special reviews (Gallagher and Chan, 2013; Arias and Cuervo, 2011; Shibutani and Yoshimori, 2014).

Some of the first experiments regarding IAPP and ALS were published by Morita et al. in 2011. They transfected the endogenous IAPP lacking cell line COS-1, an African green monkey kidney fibroblast-like cell line, with hIAPP or rIAPP and found an increased amount of autophagosomes in hIAPP-transfected cells compared to rIAPP transfection. Autophagosome quantity was detected by electron microscopy and immunoblot analysis of LC3 (microtubule-associated protein light chain 3) (Morita et al., 2011). Unbound LC3 (also called LC3-I), if initiated by certain pathways, binds to the lipid molecule phosphatidylethanolamine (PE) of the expanding phagophore, then called LC3-II, resulting in autophagosome membrane formation. Therefore, LC3-I conversion to LC3-II serves as a useful marker to measure autophagosome formation. hIAPP treated INS-1 cells, transduced INS-1 cells (rat insulinoma cell lines), transgenic rats and transgenic mice also showed an increase in autophagosome formation compared to rIAPP (Rivera et al., 2011; Shigihara et al., 2014). Rivera et al. additionally described an elevated occurrence of autophagosomes in rIAPP transduced INS 832/13 cells compared to non-transduced cells, but to a much lesser extent compared to hIAPP transduction (Rivera et al., 2011). The question arises, if this increase in autophagosomes is due to an increased autophagic flux and consequently an increased autophagosome formation or if impaired lysosomal degradation is the underlying reason.

The results adressing this question are controversial. p62, also called sequestosome 1 or SQSTM1, is an important macroautophagy receptor serving for the delivery of the substrate to the autophagosome. The substrate, which is ubiquitinated by E3, binds to p62 and the p62/substrate-complex is then uptaken by the autophagosome mediated by LC3. In hIAPP treated INS-1 cells, transduced INS 832/13 cells and transgenic mice p62 protein levels were not affected indicating that the lysosomal activity is not affected and autophagosome formation is induced (Shigihara et al., 2014). In contrast, elevated p62 protein levels were detected in hIAPP transgenic rats compared to wildtype rats. However, mRNA levels remained unchanged. This led to the different assumption that lysosomal degradation is decreased leading to an accumulation of p62. hIAPP transgenic mice showed an increase of both p62 mRNA and protein levels compared to rIAPP. However, mRNA level increase was less distinctive than p62 protein levels. In contrast, studies with hIAPP transduced INS 832/13 revealed an increase of p62 followed by a rapid decrease after 36 and 48 h. This was in parallel accompanied by apoptosis induction leading to the hypothesis that p62 could have a possible role in β-cell survival (Rivera et al., 2011).

A possible explanation for the decreased lysosomal activity may be the ability of toxic hIAPP oligomers to disrupt cellular membranes (Engel et al., 2008; Janson et al., 1999). Lysosomal membranes could be punctured by spiky hIAPP structures leading to an increase of the lysosomal pH. Since the lysosomal enzymes show maximum activity at acidic pH, degradation of the cargo by these enzymes may consequently be disturbed (Mindell, 2012). Rupture of the lysosomal membrane may also result in the release of the enzymes leading to apoptosis (Guicciardi et al., 2004).

To test, whether autophagy plays a role in the degradation of toxic hIAPP, studies with autophagy modulators were performed. Inhibition of lysosomal proteases with pepstatin A increased hIAPP-induced apoptosis in transduced cells and IAPP content in INS 832/13 cells and human islets. Also knockout of *Atg7* leading to autophagy deficiency, resulted in increased hIAPP induced apoptosis compared to rIAPP treatment (Rivera et al., 2011, 2014; Shigihara et al., 2014). In contrast, stimulation of autophagosome formation by rapamycin, an inhibitor of the mTOR (mammalian target of rapamycin) signaling pathway, reduced hIAPP-induced apoptosis and IAPP content. These changes in intracellular IAPP content were not a consequence of an altered IAPP expression or secretion, what indicated a role of autophagy for IAPP degradation. Interestingly, insulin content was not affected. Thus, autophagy seems to regulate cellular content of IAPP. Rivera et al. further confirmed that IAPP became polyubiquitinated, possibly resulting in degradation by p62-dependent autophagy (Rivera et al., 2011, 2014). However, ubiquitination of proteins could also be a hint for degradation by 26S proteasome.

Studies from Singh et al. indicated that intracellular and extracellular IAPP may be eliminated by different clearance mechanisms. In contrast to the studies mentioned before, they treated RIN-m5F cells with hIAPP and rIAPP by adding the compounds to the culture medium. After cellular uptake, IAPP was mainly translocated into the nucleus and only small amounts colocalized with lysosomes. Autophagy inhibition with pepstatin A showed no significant change in cellular IAPP content (Singh et al., 2016).

The suggested protective role of p62 was confirmed by studies with transduced cell lines and transgenic mice, where both hIAPP and, to a lesser extent, also rIAPP immunoprecipitated with p62. Furthermore, immunoprecipitation of IAPP with p62 was exclusively found in insoluble fractions of cell lysates from hIAPP-transgenic and not from rIAPP-transgenic mice. This result, in addition to positive thioflavin S staining in p62-inclusions, revealed that p62 inclusions also contain insoluble forms of hIAPP. Further investigations reported that p62 inclusions are surrounded by components of the ALS, more precisely autophagosomes and lysosomes. This could be a hint that hIAPP and rIAPP are degraded by p62-dependent autophagy and furthermore that p62 protects the cell from toxic hIAPP species. Additional experiments reported that β-cells of hIAPP transgenic mice containing p62-positive inclusions showed less binding of the specifically oligomeric hIAPP recognizing antibody A11, confirming the protective role of p62 from toxic oligomers (Rivera et al., 2014). Knockdown of p62 with shRNA increased hIAPP-mediated apoptosis in INS 832/13 cells, whereas an overexpression of p62 led to a decrease of hIAPP-induced apoptosis (Rivera et al., 2011).

Summarized, it was shown that ALS plays a role in the clearance of amylin and therefore in diminishing the cytotoxic effects of human amylin. Since autophagic flux seems to be decreased in age (Ott et al., 2016), the detrimental cellular effects of hIAPP are likely to be exacerbated due to dysfunctional autophagy as shown in studies with chemical inactivation and induction of autophagy and p62 expression. Human amylin also seems to interfere with autophagic pathways. So far, it is unknown, if autophagosome accumulation is the consequence of an increased formation or an impaired lysosomal degradation due to hIAPP burden.

## 5. Conclusion

Current knowledge about the effects of amylin on UPS and ALS and the role of both degradation systems in amylin turnover is limited.

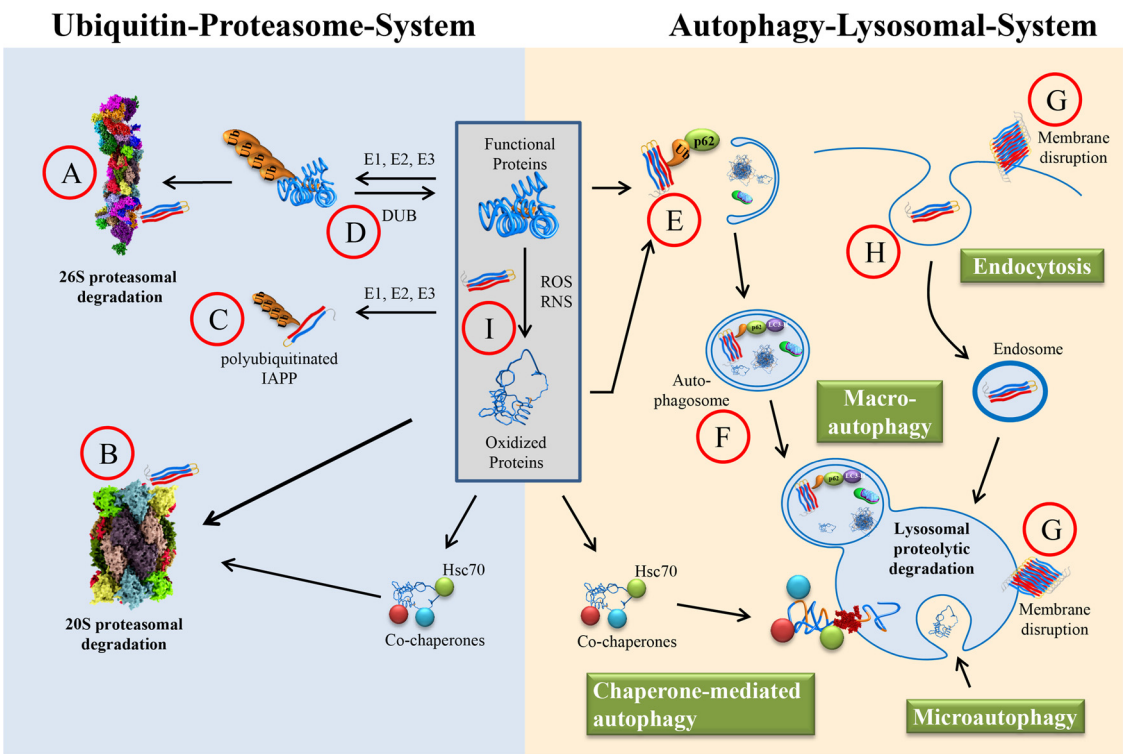


**Fig. 4.** Overview of the UPS and ALS including amylin turnover and effects.
Folded and functional proteins are degraded by the 26S proteasome in an ubiquitin-dependent way. Some proteins are rescued from degradation by DUBs. ROS-mediated damaged proteins, misfolded proteins and protein aggregates are degraded by 20S proteasome and to a lesser extent by autophagy. The p62/ubiquitin/substrate-complex binds to phagophore membrane-bound LC3-II, the autophagosome formation progresses and the autophagosome fuses with the lysosome, where cargo is being degraded (macroautophagy). In microautophagy, cytosolic substrates are directly engulfed by the lysosome. In CMA, chaperone Hsc70 and co-chaperones mediate cargo uptake into the lysosome *via* LAMP2A receptor. Autophagic cargo is degraded in lysosomes. Extracellular proteins can be taken up by endocytosis or related processes. Human amylin seems to interfere in both UPS and ALS and in turn IAPP seems to be degraded by both systems. Several Studies show that human IAPP can impact on 26S proteasome (A) or 20S proteasomal function (B). Furthermore, IAPP can be polyubiquitinated (C), but on the other side disturb the deubiquitination process (D). IAPP has impact on the cellular amount of autophagosomes (F), possibly due to an increased autophagic flux or an impaired lysosomal function. hIAPP oligomers are able to disrupt cell membranes (G), potentially leading to dysfunctional lysosomes. hIAPP molecules might be taken up by endocytotic processes (H). Among others, membrane disruption (G) or intracellular damage might be due to IAPP oligomer-triggered oxidant production (I). IAPP might be cleared by the UPS (A, B), but also by p62-dependent autophagy (E).

Nevertheless, there is evidence that UPS as well as ALS are involved in the clearance of amylin, but are also affected by amylin, especially by the human one (summarized in Fig. 4). However, the mechanisms causing disturbances in both proteolytic pathways are unknown. Studies investigating the UPS and ALS in response to hIAPP are incoherent possibly due to different methods and models used. UPS and ALS as the main proteolytic systems influence the cellular fate and therefore also the aging process. A decline of both systems is a well-known characteristic of aging. So far, it is not fully understood, if hIAPP aggregates accumulate during aging. However, it is reasonable that the age-dependent decrease of proteolytic activity has an impact on the detrimental effects of human amylin especially in β-cells and therefore on T2D pathogenesis.

## References


Akesson, B., et al., 2003. Islet amyloid polypeptide inhibits glucagon release and exerts a dual action on insulin release from isolated islets. Regul. Pept. 111 (1-3), 55–60.

Ames, B.N., Shigenaga, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. U. S. A. 90 (17), 7915–7922.

Anguiano, M., Nowak, R.J., Lansbury Jr, P.T., 2002. Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes. Biochemistry 41 (38), 11338–11343.

Arendt, C.S., Hochstrasser, M., 1997. Identification of the yeast 20S proteasome catalytic centers and subunit interactions required for active-site formation. Proceedings of the National Academy of Sciences of the United States of America 94 (14), 7156–7161.

Arias, E., Cuervo, A.M., 2011. Chaperone-mediated autophagy in protein quality control. Curr. Opin. Cell. Biol. 23 (2), 184–189.

Barth, S.W., et al., 2004. Peripheral amylin activates circumventricular organs expressing calcitonin receptor a/b subtypes and receptor-activity modifying proteins in the rat. Brain Res. 997 (1), 97–102.

Bedford, L., et al., 2010. Assembly, structure, and function of the 26S proteasome. Trends Cell Biol. 20 (7), 391–401.

Benavides, S.H., et al., 2002. Sequential histochemical studies of neuronal lipofuscin in human cerebral cortex from the first to the ninth decade of life. Arch. Gerontol. Geriatr. 34 (3), 219–231.

Betsholtz, C., et al., 1989. Islet amyloid polypeptide (Iapp) - Cdna cloning and identification of an amyloidogenic region associated with the species-specific occurrence of age-related diabetes-mellitus. Exp. Cell Res. 183 (2), 484–493.

Bhogal, R., Smith, D.M., Bloom, S.R., 1992. Investigation and characterization of binding sites for islet amyloid polypeptide in rat membranes. Endocrinology 130 (2), 906–913.

Bishop, P., Rocca, D., Henley, J.M., 2016. Ubiquitin C-terminal hydrolase L1 (UCH-L1): structure, distribution and roles in brain function and dysfunction. Biochem. J. 473 (16), 2453–2462.

Brain, S.D., et al., 1990. The demonstration of vasodilator activity of pancreatic amylin amide in the rabbit. Am. J. Pathol. 136 (3), 487–490.

Broderick, C.L., et al., 1991. Human and rat amylin have no effects on insulin secretion in isolated rat pancreatic islets. Biochem. Biophys. Res. Commun. 177 (3), 932–938.

Bulteau, A.L., Petropoulos, I., Friguet, B., 2000. Age-related alterations of proteasome structure and function in aging epidermis. Exp. Gerontol. 35 (6–7), 767–777.

Bulteau, A.L., Szweda, L.I., Friguet, B., 2002. Age-dependent declines in proteasome activity in the heart. Arch. Biochem. Biophys. 397 (2), 298–304.

Carnagarin, R., Dharmarajan, A.M., Dass, C.R., 2015. Molecular aspects of glucose homeostasis in skeletal muscle–a focus on the molecular mechanisms of insulin resistance. Mol. Cell. Endocrinol. 417, 52–62.

Casas, S., et al., 2007. Impairment of the ubiquitin-proteasome pathway is a downstream endoplasmic reticulum stress response induced by extracellular human islet amyloid polypeptide and contributes to pancreatic beta-cell apoptosis. Diabetes 56 (9), 2284–2294.

Chondrogianni, N., et al., 2003. Central role of the proteasome in senescence and survival of human fibroblasts: induction of a senescence-like phenotype upon its inhibition and resistance to stress upon its activation. J. Biol. Chem. 278 (30), 28026–28037.

Chondrogianni, N., et al., 2015. 20S proteasome activation promotes life span extension and resistance to proteotoxicity in Caenorhabditis elegans. FASEB J. 29 (2), 611–622.
Christopoulos, G., et al., 1999. Multiple amylin receptors arise from receptor activity-modifying protein interaction with the calcitonin receptor gene product. Mol. Pharmacol. 56 (1), 235–242.
Clague, M.J., Heride, C., Urbe, S., 2015. The demographics of the ubiquitin system. Trends Cell. Biol. 25 (7), 417–426.
Clementi, G., et al., 1996. Amylin given by central or peripheral routes decreases gastric emptying and intestinal transit in the rat. Experientia 52 (7), 677–679.
Conconi, M., et al., 1998. Protection from oxidative inactivation of the 20S proteasome by heat-shock protein 90. Biochem. J. 333 (Pt 2), 407–415.
Costes, S., et al., 2011. Beta-cell dysfunctional ERAD/ubiquitin/proteasome system in type 2 diabetes mediated by islet amyloid polypeptide-induced UCH-L1 deficiency. Diabetes 60 (1), 227–238.
Couve, E., Schmachtenberg, O., 2011. Autophagic activity and aging in human odontoblasts. J. Dent. Res. 90 (4), 523–528.
Couve, E., Osorio, R., Schmachtenberg, O., 2012. Mitochondrial autophagy and lipofuscin accumulation in aging odontoblasts. J. Dent. Res. 91 (7), 696–701.
Davies, K.J., 1995. Oxidative stress: the paradox of aerobic life. Biochem. Soc. Symp. 61, 1–31.
Degano, P., et al., 1993. Amylin inhibits glucose-induced insulin secretion in a dose-dependent manner. Study in the perfused rat pancreas. Regul. Pept. 43 (1-2), 91–96.
Dengjel, J., et al., 2005. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. Proc. Natl. Acad. Sci. U. S. A. 102 (22), 7922–7927.
Dobson, C.M., 1999. Protein misfolding, evolution and disease. Trends Biochem. Sci. 24 (9), 329–332.
Dowling, S.D., Macian, F., 2018. Autophagy and T cell metabolism. Cancer Lett. 419, 20–26.
Dues, D.J., et al., 2016. Aging causes decreased resistance to multiple stresses and a failure to activate specific stress response pathways. Aging 8 (4), 777–795.
Engel, M.F., et al., 2006. Islet amyloid polypeptide inserts into phospholipid monolayers as monomer. J. Mol. Biol. 356 (3), 783–789.
Engel, M.F., et al., 2008. Membrane damage by human islet amyloid polypeptide through fibril growth at the membrane. Proc. Natl. Acad. Sci. U. S. A. 105 (16), 6033–6038.
Ferrier, G.J., et al., 1989. Expression of the rat amylin (IAPP/DAP) gene. J. Mol. Endocrinol. 3 (1), R1–4.
Ferrington, D.A., Husom, A.D., Thompson, L.V., 2005. Altered proteasome structure, function, and oxidation in aged muscle. Faseb J. 19 (6), 644–646.
Gallagher, L.E., Chan, E.Y., 2013. Early signalling events of autophagy. Essays Biochem. 55, 1–15.
Gedulin, B.R., Rink, T.J., Young, A.A., 1997. Dose-response for glucagonostatic effect of amylin in rats. Metabolism 46 (1), 67–70.
German, M.S., et al., 1992. The insulin and islet amyloid polypeptide genes contain similar cell-specific promoter elements that bind identical Beta-cell nuclear-complexes. Mol. Cell. Biol. 12 (4), 1777–1788.
Giaccone, G., et al., 2011. Lipofuscin hypothesis of Alzheimer's disease. Dement. Geriatr. Cogn. Dis. Extra 1 (1), 292–296.
Grimm, S., et al., 2011. Protein oxidative modifications in the ageing brain: consequence for the onset of neurodegenerative disease. Free Radic Res. 45 (1), 73–88.
Groll, M., et al., 2000. A gated channel into the proteasome core particle. Nat. Struct. Biol. 7 (11), 1062–1067.
Grune, T., et al., 2001. Age-related changes in protein oxidation and proteolysis in mammalian cells. J. Gerontol. A Biol. Sci. Med. Sci. 56 (11), B459–67.
Guarente, L., Kenyon, C., 2000. Genetic pathways that regulate ageing in model organisms. Nature 408 (6809), 255–262.
Guicciardi, M.E., Leist, M., Gores, G.J., 2004. Lysosomes in cell death. Oncogene 23 (16), 2881–2890.
Halliwell, B., Chirico, S., 1993. Lipid peroxidation: its mechanism, measurement, and significance. Am. J. Clin. Nutr. 57 (5 Suppl), 715S–724S discussion 724S-725S.
Harman, D., 1989. Lipofuscin and ceroid formation: the cellular recycling system. Adv. Exp. Med. Biol. 266, 3–15.
Hayflick, L., 2000. The future of ageing. Nature 408 (6809), 267–269.
Höhn, A., et al., 2010. Lipofuscin-bound iron is a major intracellular source of oxidants: role in senescent cells. Free Radic. Biol. Med. 48 (8), 1100–1108.
Höhn, A., et al., 2011. Lipofuscin inhibits the proteasome by binding to surface motifs. Free Radic. Biol. Med. 50 (5), 585–591.
Höhn, A., et al., 2012. Lipofuscin is formed independently of macroautophagy and lysosomal activity in stress-induced prematurely senescent human fibroblasts. Free Radic. Biol. Med. 53 (9), 1760–1769.
Höhn, A., et al., 2017. Happily (n)ever after: aging in the context of oxidative stress, proteostasis loss and cellular senescence. Redox Biol. 11, 482–501.
Höhn, A., Grune, T., 2013. Lipofuscin: formation, effects and role of macroautophagy. Redox Biol. 1, 140–144.
Hoppener, J.W., Ahren, B., Lips, C.J., 2000. Islet amyloid and type 2 diabetes mellitus. N. Engl. J. Med. 343 (6), 411–419.
Hull, R.L., et al., 2004. Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. J. Clin. Endocrinol. Metab. 89 (8), 3629–3643.
Imai, J., et al., 2003. The molecular chaperone Hsp90 plays a role in the assembly and maintenance of the 26S proteasome. Embo J. 22 (14), 3557–3567.
Jaikaran, E.T., et al., 2001. Identification of a novel human islet amyloid polypeptide beta-sheet domain and factors influencing fibrillogenesis. J. Mol. Biol. 308 (3), 515–525.
Jaikaran, E.T., Clark, A., 2001. Islet amyloid and type 2 diabetes: from molecular misfolding to islet pathophysiology. Biochim. Biophys. Acta 1537 (3), 179–203.
Janson, J., et al., 1999. The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. Diabetes 48 (3), 491–498.
Johnson, K.H., et al., 1992. Islet amyloid polypeptide: mechanisms of amyloidogenesis in the pancreatic islets and potential roles in diabetes mellitus. Lab. Invest. 66 (5), 522–535.
Johnson, T.E., et al., 2000. Gerontogenes mediate health and longevity in nematodes through increasing resistance to environmental toxins and stressors. Exp. Gerontol. 35 (6–7), 687–694.
Jolly, R.D., Palmer, D.N., Dalefield, R.R., 2002. The analytical approach to the nature of lipofuscin (age pigment). Arch. Gerontol. Geriatr. 34 (3), 205–217.
Jung, T., et al., 2006. Intracellular distribution of oxidized proteins and proteasome in HT22 cells during oxidative stress. Free Radical Biol. Med. 40 (8), 1303–1312.
Jung, T., Catalgol, B., Grune, T., 2009. The proteasomal system. Mol. Asp. Med. 30 (4), 191–296.
Kahn, S.E., et al., 1990. Evidence of cosecretion of islet amyloid polypeptide and insulin by beta-cells. Diabetes 39 (5), 634–638.
Kahn, S.E., et al., 1991. Glucose stimulates and potentiates islet amyloid polypeptide secretion by the B-cell. Horm. Metab. Res. 23 (12), 577–580.
Kapphahn, R.J., Bigelow, E.J., Ferrington, D.A., 2007. Age-dependent inhibition of proteasome chymotrypsin-like activity in the retina. Exp. Eye Res. 84 (4), 646–654.
Kogire, M., et al., 1991. Inhibitory action of islet amyloid polypeptide and calcitonin gene-related peptide on release of insulin from the isolated perfused rat pancreas. Pancreas 6 (4), 459–463.
Kumar, P., et al., 2008. Effect of dehydroepiandrosterone (DHEA) on monoamine oxidase activity, lipid peroxidation and lipofuscin accumulation in aging rat brain regions. Biogerontology 9 (4), 235–246.
Leckstrom, A., et al., 1999. Islet amyloid polypeptide and insulin relationship in a longitudinal study of the genetically obese (ob/ob) mouse. Pancreas 18 (3), 266–273.
Levine, R.L., Stadtman, E.R., 2001. Oxidative modification of proteins during aging. Exp. Gerontol. 36 (9), 1495–1502.
Lopez-Otin, C., et al., 2013. The hallmarks of aging. Cell 153 (6), 1194–1217.
Luca, S., et al., 2007. Peptide conformation and supramolecular organization in amylin fibrils: constraints from solid-state NMR. Biochemistry 46 (47), 13505–13522.
Lutz, T.A., 2005. Pancreatic amylin as a centrally acting satiating hormone. Curr. Drug. Targets 6 (2), 181–189.
Madeo, F., et al., 2015. Essential role for autophagy in life span extension. J. Clin. Invest. 125 (1), 85–93.
Marshall, K.E., et al., 2014. The relationship between amyloid structure and cytotoxicity. Prion 8 (2).
Marzban, L., et al., 2004. Role of beta-cell prohormone convertase (PC) 1/3 in processing of pro-islet antyloid polypeptide. Diabetes 53 (1), 141–148.
Masters, S.L., et al., 2010. Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes. Nat. Immunol. 11 (10), 897–904.
Mattson, M.P., Goodman, Y., 1995. Different amyloidogenic peptides share a similar mechanism of neurotoxicity involving reactive oxygen species and calcium. Brain Res. 676 (1), 219–224.
McLatchie, L.M., et al., 1998. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 393 (6683), 333–339.
Middleton, C.T., et al., 2012. Two-dimensional infrared spectroscopy reveals the complex behaviour of an amyloid fibril inhibitor. Nat. Chem. 4 (5), 355–360.
Mindell, J.A., 2012. Lysosomal acidification mechanisms. Annu. Rev. Physiol. 74, 69–86.
Mirzabekov, T.A., Lin, M.C., Kagan, B.L., 1996. Pore formation by the cytotoxic islet amyloid peptide amylin. J. Biol. Chem. 271 (4), 1988–1992.
Miyazato, M., et al., 1991. Identification and characterization of islet amyloid polypeptide in mammalian gastrointestinal tract. Biochem. Biophys. Res. Commun. 181 (1), 293–300.
Mizushima, N., Levine, B., 2010. Autophagy in mammalian development and differentiation. Nat. Cell Biol. 12 (9), 823–830.
Morita, S., et al., 2011. Autophagy protects against human islet amyloid polypeptide-associated apoptosis. J. Diabetes Invest. 2 (1), 48–55.
Muff, R., et al., 1999. Amylin receptor is revealed following co-transfection of a calcitonin receptor with receptor activity modifying proteins-1or-3. Endocrinology 140 (6), 2924–2927.
Mukherjee, P., et al., 2009. Monitoring the accumulation of lipofuscin in aging murine eyes by fluorescence spectroscopy. Photochem. Photobiol. 85 (1), 234–238.
Mukherjee, A., et al., 2015. Type 2 diabetes as a protein misfolding disease. Trends Mol. Med. 21 (7), 439–449.
Mulder, H., et al., 1994. Islet amyloid polypeptide is expressed in endocrine-cells of the gastric-mucosa in the rat and mouse. Gastroenterology 107 (3), 712–719.
Mulder, H., et al., 1995. Islet amyloid polypeptide (amylin) is expressed in sensory neurons. J. Neurosci. 15 (11), 7625–7632.
Mulder, H., et al., 1997. Islet amyloid polypeptide in the gut and pancreas: localization, ontogeny and gut motility effects. Peptides 18 (6), 771–783.
Mulder, H., Ahren, B., Sundler, F., 1996. Islet amyloid polypeptide and insulin gene expression are regulated in parallel by glucose in vivo in rats. Am. J. Physiol. 271 (Dec. (6 Pt 1)), E1008–1014.
Nicholl, C.G., et al., 1992. Extra-pancreatic expression of the rat islet amyloid polypeptide (amylin) gene. J. Mol. Endocrinol. 9 (2), 157–163.
O'Brien, T.D., Westermark, P., Johnson, K.H., 1990. Islet amyloid polypeptide (IAPP) does not inhibit glucose-stimulated insulin secretion from isolated perfused rat pancreas. Biochem. Biophys. Res. Commun. 170 (3), 1223–1228.
Okada, K., et al., 1999. 4-Hydroxy-2-nonenal-mediated impairment of intracellular proteolysis during oxidative stress. Identification of proteasomes as target molecules. J. Biol. Chem. 274 (34), 23787–23793.
Oliver, C.N., et al., 1987. Age-related changes in oxidized proteins. J. Biol. Chem. 262 (12), 5488–5491.
Orlowski, M., Wilk, S., 2000. Catalytic activities of the 20 S proteasome, a multicatalytic

proteinase complex. Arch. Biochem. Biophys. 383 (1), 1–16.
Ott, C., et al., 2016. Reduced autophagy leads to an impaired ferritin turnover in senescent fibroblasts. Free Radic. Biol. Med. 101, 325–333.
Panagiotidis, G., et al., 1992. Homologous islet amyloid polypeptide: effects on plasma levels of glucagon, insulin and glucose in the mouse. Diabetes Res. Clin. Pract. 18 (3), 167–171.
Paulsson, J.F., et al., 2006. Intracellular amyloid-like deposits contain unprocessed pro-islet amyloid polypeptide (proIAPP) in beta cells of transgenic mice overexpressing the gene for human IAPP and transplanted human islets. Diabetologia 49 (6), 1237–1246.
Perse, M., Injac, R., Erman, A., 2013. Oxidative status and lipofuscin accumulation in urothelial cells of bladder in aging mice. PLoS One 8 (3), e59638.
Pettersson, M., Ahren, B., 1990. Failure of islet amyloid polypeptide to inhibit basal and glucose-stimulated insulin secretion in model experiments in mice and rats. Acta Physiol. Scand. 138 (3), 389–394.
Powell, S.R., et al., 2005. Aggregates of oxidized proteins (lipofuscin) induce apoptosis through proteasome inhibition and dysregulation of proapoptotic proteins. Free Radic. Biol. Med. 38 (8), 1093–1101.
Prigge, S.T., et al., 1997. Amidation of bioactive peptides: the structure of peptidylglycine alpha-hydroxylating monooxygenase. Science 278 (5341), 1300–1305.
Qian, M.J., Fang, X.C., Wang, X.D., 2017. Autophagy and inflammation. Clin. Transl. Med. 6.
Rabl, J., et al., 2008. Mechanism of gate opening in the 20S proteasome by the proteasomal ATPases. Mol. Cell. 30 (3), 360–368.
Ristic, G., Tsou, W.L., Todi, S.V., 2014. An optimal ubiquitin-proteasome pathway in the nervous system: the role of deubiquitinating enzymes. Front. Mol. Neurosci. (7), 72.
Rivera, J.F., et al., 2011. Human-IAPP disrupts the autophagy/lysosomal pathway in pancreatic beta-cells: protective role of p62-positive cytoplasmic inclusions. Cell Death Differ. 18 (3), 415–426.
Rivera, J.F., et al., 2014. Autophagy defends pancreatic beta cells from human islet amyloid polypeptide-induced toxicity. J. Clin. Invest. 124 (8), 3489–3500.
Roth, J.D., et al., 2007. Combination therapy with amylin and peptide YY[3-36] in obese rodents: anorexigenic synergy and weight loss additivity. Endocrinology 148 (12), 6054–6061.
Roth, J.D., et al., 2008. Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc. Natl. Acad. Sci. U. S. A. 105 (20), 7257–7262.
Rushing, P.A., et al., 2000a. Amylin: a novel action in the brain to reduce body weight. Endocrinology 141 (2), 850–853.
Rushing, P.A., et al., 2000b. Amylin and insulin interact to reduce food intake in rats. Horm. Metab. Res. 32 (2), 62–65.
Sanke, T., et al., 1988. An islet amyloid peptide is derived from an 89-amino acid precursor by proteolytic processing. J. Biol. Chem. 263 (33), 17243–17246.
Schmucker, D.L., Sachs, H., 2002. Quantifying dense bodies and lipofuscin during aging: a morphologist's perspective. Arch. Gerontol. Geriatr. 34 (3), 249–261.
Schubert, D., et al., 1995. Amyloid peptides are toxic via a common oxidative mechanism. Proc. Natl. Acad. Sci. U. S. A. 92 (6), 1989–1993.
Settembre, C., et al., 2013. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. Nat. Rev. Mol. Cell Biol. 14 (5), 283–296.
Shibutani, S.T., Yoshimori, T., 2014. A current perspective of autophagosome biogenesis. Cell. Res. 24 (1), 58–68.
Shigihara, N., et al., 2014. Human IAPP-induced pancreatic beta cell toxicity and its regulation by autophagy. J. Clin. Invest. 124 (8), 3634–3644.
Sies, H., 1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82 (2), 291–295.
Singh, S., et al., 2016. Proteasome regulates turnover of toxic human amylin in pancreatic cells. Biochem. J. 473, 2655–2670.
Sitte, N., et al., 2000a. Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. FASEB J. 14 (11), 1490–1498.
Sitte, N., et al., 2000b. Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part II–aging of nondividing cells. Faseb J. 14 (15), 2503–2510.
Sitte, N., et al., 2000c. Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part I–effects of proliferative senescence. Faseb J. 14 (15), 2495–2502.
Smith, C.D., et al., 1991. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc. Natl. Acad. Sci. U. S. A. 88 (23), 10540–10543.
Steiner, D.F., et al., 1993. New molecular perspectives in islet hormone biosynthesis. Biochem. Soc. Trans. 21 (1), 139–142.
Su, Y., et al., 2012. The occurrence of islet amyloid polypeptide amyloidosis in Japanese subjects. Pancreas 41 (6), 971–973.
Suter, M., et al., 2000. Age-related macular degeneration. The lipofusion component N-retinyl-N-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. J. Biol. Chem. 275 (50), 39625–39630.
Tai, H.C., Schuman, E.M., 2008. Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction. Nat. Rev. Neurosci. 9 (11), 826–838.
Takada, K., et al., 1996. Islet amyloid polypeptide/amylin contents in pancreas change with increasing age in genetically obese and diabetic mice. Diabetes Res. Clin. Pract. 33 (3), 153–158.
Terman, A., Brunk, U.T., 2004. Lipofuscin. Int. J. Biochem. Cell. Biol. 36 (8), 1400–1404.
Tilakaratne, N., et al., 2000. Amylin receptor phenotypes derived from human calcitonin receptor/RAMP coexpression exhibit pharmacological differences dependent on receptor isoform and host cell environment. J. Pharmacol. Exp. Ther. 294 (1), 61–72.
Tjernberg, L.O., et al., 1999. Amyloid beta-peptide polymerization studied using fluorescence correlation spectroscopy. Chem. Biol. 6 (1), 53–62.
Tosato, M., et al., 2007. The aging process and potential interventions to extend life expectancy. Clin. Interv. Aging 2 (3), 401–412.
Toshimori, H., et al., 1990. Islet amyloid polypeptide (iapp) in the gastrointestinal-tract and pancreas of man and rat. Cell Tissue Res. 262 (3), 401–406.
Trevaskis, J.L., et al., 2010a. Interaction of leptin and amylin in the long-term maintenance of weight loss in diet-induced obese rats. Obesity (Silver Spring) 18 (1), 21–26.
Trevaskis, J.L., Parkes, D.G., Roth, J.D., 2010b. Insights into amylin-leptin synergy. Trends Endocrinol. Metab. 21 (8), 473–479.
Tsakiri, E.N., et al., 2013. Diet-derived advanced glycation end products or lipofuscin disrupts proteostasis and reduces life span in Drosophila melanogaster. Free Radic. Biol. Med. 65, 1155–1163.
Turek, V.F., et al., 2010. Mechanisms of amylin/leptin synergy in rodent models. Endocrinology 151 (1), 143–152.
Ueda, T., et al., 2001. Expression of receptor-activity modifying protein (RAMP) mRNAs in the mouse brain. Mol. Brain Res. 93 (1), 36–45.
Villa, I., et al., 1997. Effects of amylin on human osteoblast-like cells. Peptides 18 (4), 537–540.
Wang, Z.L., et al., 1993. Influence of islet amyloid polypeptide and the 8-37 fragment of islet amyloid polypeptide on insulin release from perifused rat islets. Diabetes 42 (2), 330–335.
Wang, J., et al., 2001. The prohormone convertase enzyme 2 (PC2) is essential for processing pro-islet amyloid polypeptide at the NH2-terminal cleavage site. Diabetes 50 (3), 534–539.
Wang, R.C., Levine, B., 2010. Autophagy in cellular growth control. FEBS Lett. 584 (7), 1417–1426.
Wehmer, M., Sakata, E., 2016. Recent advances in the structural biology of the 26S proteasome. Int. J. Biochem. Cell. Biol. (79), 437–442.
Westermark, P., et al., 1986. A novel peptide in the calcitonin Gene related peptide family as an amyloid fibril protein in the endocrine pancreas. Biochem. Biophys. Res. Commun. 140 (3), 827–831.
Westermark, P., et al., 1987. Amyloid fibrils in human insulinoma and islets of langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islet cells. Proc. Natl. Acad. Sci. U. S. A. 84 (11), 3881–3885.
Westermark, P., et al., 1990. Islet amyloid polypeptide: pinpointing amino acid residues linked to amyloid fibril formation. Proc. Natl. Acad. Sci. U. S. A. 87 (13), 5036–5040.
Westermark, P., 2005. Aspects on human amyloid forms and their fibril polypeptides. Febs J. 272 (23), 5942–5949.
Wickbom, J., et al., 2008. Gastric emptying in response to IAPP and CCK in rats with subdiaphragmatic afferent vagotomy. Regul. Pept. 148 (1–3), 21–25.
Wielinga, P.Y., et al., 2010. Central amylin acts as an adiposity signal to control body weight and energy expenditure. Physiol. Behav. 101 (1), 45–52.
Wielinga, P.Y., Alder, B., Lutz, T.A., 2007. The acute effect of amylin and salmon calcitonin on energy expenditure. Physiol. Behav. 91 (2-3), 212–217.
Wiltzius, J.J., et al., 2008. Atomic structure of the cross-beta spine of islet amyloid polypeptide (amylin). Protein Sci. 17 (9), 1467–1474.
Wolf, G., 2003. Lipofuscin and macular degeneration. Nutr. Rev. 61 (10), 342–346.
Wookey, P.J., Cao, Z., Cooper, M.E., 1998. Interaction of the renal amylin and renin-angiotensin systems in animal models of diabetes and hypertension. Min. Electrolyte Metab. 24 (6), 389–399.
Young, A., 2005. Inhibition of glucagon secretion. Adv. Pharmacol. 52, 151–171.
Young, A.A., Gedulin, B.R., Rink, T.J., 1996. Dose-responses for the slowing of gastric emptying in a rodent model by glucagon-like peptide (7-36) NH2, amylin, cholecystokinin, and other possible regulators of nutrient uptake. Metabolism 45 (1), 1–3.
Zaidi, M., et al., 1990. Amylin-amide: a new bone-conserving peptide from the pancreas. Exp. Physiol. 75 (4), 529–536.
Zhang, S., et al., 1999. Induction of apoptosis by human amylin in RINm5F islet beta-cells is associated with enhanced expression of p53 and p21WAF1/CIP1. FEBS Lett. 455 (3), 315–320.
Zhang, S., et al., 2002. Increased expression and activation of c-Jun contributes to human amylin-induced apoptosis in pancreatic islet beta-cells. J. Mol. Biol. 324 (2), 271–285.
Zhang, S., et al., 2008. Fas-associated death receptor signaling evoked by human amylin in islet beta-cells. Diabetes 57 (2), 348–356.
Zhang, Y., et al., 2017. The role of ubiquitin proteasomal system and autophagy-lysosome pathway in Alzheimer's disease. Rev. Neurosci. 28 (8), 861–868.
Zhang, H., Davies, K.J.A., Forman, H.J., 2015. Oxidative stress response and Nrf2 signaling in aging. Free Radic. Biol. Med. 88 (Pt B), 314–336.