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Role of autophagy in the control of food intake

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Main field:

Biochemistry and molecular biology

Secondary fields:

Physiology and physiopathology
Nutrition and food science

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Abbreviations

ACC. Acetyl CoA carboxylase

ACTH. Proopiomelanocortin (in humans)

AgRP. Agouti-related protein

AKT. RAC-alpha serine/threonine-protein kinase

Alfy. Autophagy-linked FYVE protein

AMP. Adenosine monophosphate

AMPK. AMP-activated protein kinase

ARC. Arcuate nucleus

Atg. Autophagy-related genes

ATP. Adenosine triphosphate

CMA. Chaperone-mediated autophagy

CNS. Central nervous system

CPE. Carboxypeptidase E

CPT1. Carnitine palmitoyltransferase 1

DFCP1. Double FYVE-containing protein 1

EGFR. Epidermal growth factor receptor

ER. Endoplasmic reticulum

FFA. Free fatty acids

FIP2000. Fusion-inhibiting peptide 2000

FoxO. Forkhead box protein O

GABA. Gamma-amino-butyric acid

GTP. Guanosine triphosphate

HFD. High Fat Diet

Hsc70. Constitutive heat shock protein 70

JAK. Janus kinase

LAMP-2A. Lysosomal-associated membrane protein 2A

LC3. Microtubule-associated protein light chain 3

LCFA. Long chain fatty acids

MTMR. Myotubularin-related phosphatase

MTOR. Mammalian target of rapamycin

NPY. Neuropeptide y

PI3K. Phosphatidylinositol-3-kinase

PI3P. Phosphatidylinositol-3-phosphate

POMC. Proopiomelanocortin

ROS. Reactive oxygen species

SKG6. Suppressor of lethality of Kex2 Gas1 6

SQSTM. Sequestosome

STAT. Signal transducer and activator of transcription

TECPR1. Tectonin beta-propeller repeat-containing protein 1

TFEB. Transcription factor E-box

TNF. Tumor necrosis factor

TRAF6. TNF receptor-associated factor 6

TSC2. Tuberous sclerosis complex 2

ULK1. Unc-51-like kinase 1

UPR. Unfolded protein response

Vps. Vacuolar protein sorting-associated protein

WIPI. WD repeat domain phosphoinositide-interacting protein 2

α-MSH. Alpha-melanocyte stimulating hormone

Abstract

Autophagy is an intracellular pathway which has been well characterised since many years ago. It is present in all cell types and tissues and it is implicated in cell quality control removing from its cytosol those cellular components that could damage the cell. It is also implicated in the control of cellular energy balance and provides the cell a source of nutrients and energy during starvation. Recently, some research groups have been studying the possible role of autophagy in the hypothalamic control of food intake and energy balance. The results obtained by these groups seemed to be all in the same direction. They affirmed that autophagy had an essential role in the control of food intake. However, a recent study questions previous results, exposing that it is not autophagy but autophagic proteins which are implicated in food intake regulation by a non-autophagic mechanism. The role of autophagy in food intake is under debate and needs to be further studied.

L'autofàgia és una via cel·lular que ha estat ben caracteritzada al llarg dels anys. Està present en tots els teixits i regula el correcte funcionament de les cèl·lules participant en el control de qualitat intracel·lular eliminant aquells components cel·lulars que poden resulta'ls-hi nocius. També regula el balanç energètic a nivell cel·lular, proporcionant una font de nutrients i energia quan la cèl·lula està en condicions de desnutrició i no pot obtenir-los de l'exterior. Recentment, s'ha estudiat el possible paper de l'autofàgia en la regulació del balanç energètic i la ingesta a l'hipotàlem. Els resultats que es van obtenir anaven en la mateixa direcció afirmant que l'autofàgia tenia un paper essencial en el control de la regulació de la ingesta. Malgrat això, un estudi recent qüestiona els resultats previs, exposant que no és l'autofàgia, sinó les proteïnes que hi participen les que estan implicades en la regulació de la ingesta mitjançant un mecanisme no autofàgic. El possible paper de l'autofàgia en la regulació de la ingesta requereix ser estudiat amb més profunditat.

La autofagia es una vía celular que ha sido bien caracterizada a lo largo de los años. Está presente en todos los tejidos y regula el correcto funcionamiento de las células participando en el control de calidad intracelular y eliminando aquellos componentes celulares que pueden resultarles nocivos. También regula el balance energético a nivel celular, proporcionando una fuente de nutrientes y energía cuando la célula está en condiciones de desnutrición y no puede obtenerlos del exterior. Recientemente, se ha estudiado el posible papel de la autofagia en la regulación del balance energético y de la ingesta en el hipotálamo. Los resultados que se obtuvieron iban en la misma dirección afirmando que la autofagia tenía un papel esencial en el control de la regulación de la ingesta. Sin embargo, un estudio reciente cuestiona los

resultados previos, exponiendo que no es la autofagia, sino las proteínas que participan en su desarrollo, las que están implicadas en la regulación de la ingesta mediante un mecanismo no autofágico. El posible papel de la autofagia en la regulación de la ingesta requiere ser estudiado con más profundidad.

1. Introduction

Autophagy is a cellular catabolic process implicated in the degradation of intracellular components, such as proteins or organelles among others, in lysosomes to recycle the molecules of which these are constituted[1,2].

Three basic forms of autophagy coexist in cells at the same time (Fig. 1), they are known as macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Macroautophagy consists in the formation of a double membrane in the cytoplasm that engulfs and sequestrates cytosolic regions forming vesicles around them, then these vesicles deliver its cargo material into the lysosomes to be degraded. Microautophagy consists in the uptake of cytosolic components by the formation of invaginations at the surface of the lysosome membrane. Finally, CMA consists in the recruitment of soluble proteins with a specific domain of five amino acids that makes them amenable to degradation through this pathway. The cytosolic chaperone Hsc70 recognizes this domain and targets it into the surface of lysosomes where interacts with LAMP-2A that induces the formation of a translocation complex that helps the protein to reach the lysosomal lumen. In contrast to other types of autophagy, in CMA proteins must be completely unfolded before reaching lysosomal lumen and they are delivered there one by one[3,4,5].

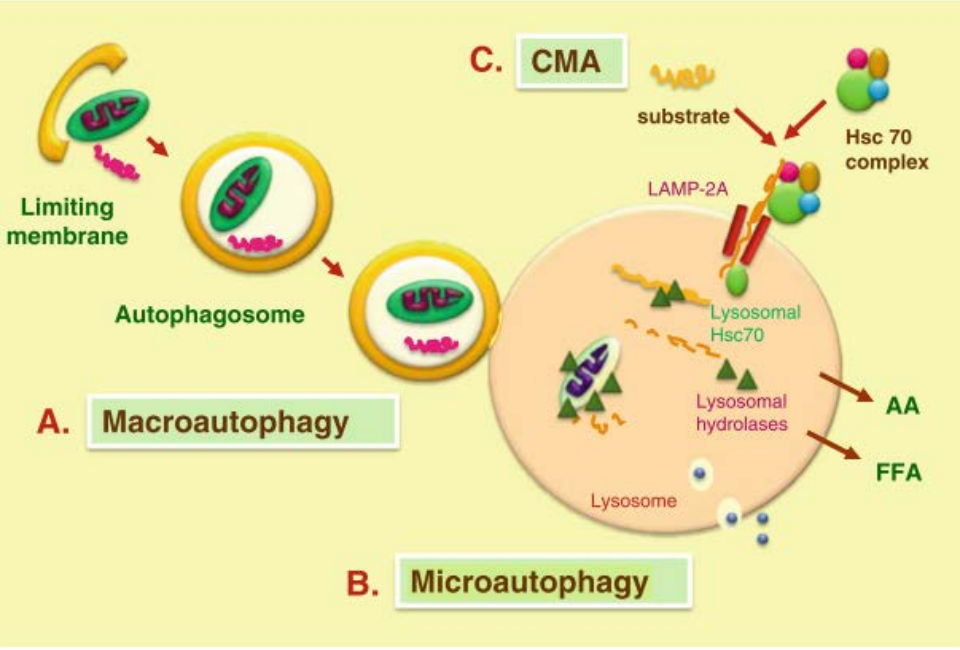

Figure 1. Types of Autophagy. A) Macroautophagy. B) Microautophagy. C) Chaperone-mediated autophagy (CMA). (Figure adapted from Ref. 4)

All three contribute to the total autophagic activity, however the main form of autophagy is macroautophagy. For this reason, the term “autophagy” usually indicates macroautophagy unless otherwise specified[6].

The basic mechanism in all of them is the sequestration of the cargo material and the final result is the delivery of cytosolic components into lysosomes for their degradation. Nevertheless, mechanisms implicated in each pathway, its regulations and the subset of genes and proteins that act as effectors and modulators in each of them are different. The main difference between macroautophagy and other two forms of autophagy lays in the sequestration of cargo material. While during macroautophagy there is a previous sequestration of the cargo material by a double membrane in the cytosol and subsequently the cargo material is delivered to lysosomes by fusion; during microautophagy and CMA the delivery of the cargo is accomplished directly from the cytosol to lysosomes[4].

Macroautophagy (hereafter referred to as autophagy) is a constitutive process that functions as a homeostatic mechanism being implicated in different cell functions. It provides an alternative source of nutrients and energy to the cell, especially during starvation. It is also important the involvement of autophagy in cellular defence, participating with innate and acquired immunity by recognizing pathogens as a degradation component[3]. In addition, it has an important contribution to cellular quality control preventing toxicity associated with accumulation of abnormal proteins or damaged organelles by removing them from the cytoplasm[2]. For this reason, alterations in autophagy have an important role in several human disorders such as cancer or neurodegenerative diseases, among others[2,7]. It has also been observed a special role of autophagy in cellular metabolism and storage of cellular lipids[1]. Finally, recent studies has shown a special role of autophagy in the regulation of food intake[8].

2. Objectives

The main objective of this academic work is to analyse how autophagy is involved in food intake regulation.

In order to achieve this objective, several secondary objectives have been proposed:

1. To analyse molecular mechanisms of autophagy. How the autophagic pathways work, which proteins are implicated and how this route is regulated.

2. To identify what kind of nutrients are implicated in regulation of autophagy, in order to understand how they could affect to autophagy and how they could influence in food intake regulation.

3. To know the main mechanisms of food intake in the hypothalamus and to analyse which is the role of autophagy in these mechanisms.

4. To identify which human disorders are related with the alteration of autophagy in the hypothalamus.

3. Materials and methods

This revision work entitled “Role of autophagy in the control of food intake” is based in the collection of the information exposed in previous review articles and original research articles. The bibliographic search has been started by searching the terms *autophagy* and *macroautophagy* in the database of the *National Library of Medicine* “PubMed” (http://www.ncbi.nlm.nih.gov/pubmed), from which it is possible to access to prestigious publications from the journals *Nature, Science* or *Cell* among others. This first search has given some global information about different forms of autophagy and the molecular mechanisms which are implicated, concretely, in the form of macroautophagy. The review articles consulted have offered some new sources, original articles that provide information about how macroautophagy influences over the regulation of food intake and about those pathologies or disorders derived from an alteration of autophagy in relation to food intake.

3.1. Integrating educational fields

This review work integrates contributions from different educational fields. During its development knowledge of biochemistry and molecular biology, nutrition and food science and physiology and physiopathology have been integrated.

- **Biochemistry and molecular biology**: Is the main field of the review work. It is used to define the molecular process of autophagy, how the steps of this pathway are developed within the cell, which are the proteins implicated in these steps, how they act and which are the complexes and mechanisms that regulate this metabolic pathway. This field is also used to analyse the mechanisms implicated in food intake regulation and its possible relation with autophagy.
- **Nutrition and food science:** To understand a possible role of autophagy in cellular energy homeostasis is necessary to describe how macronutrients: lipids, carbohydrates and proteins are implicated in autophagic mechanisms.

- **Physiology and physiopathology:** Through the physiological field and with molecular biology experience, food intake in hypothalamus can be explained with detail. Physiopathology field provides the knowledge to understand how an autophagic failure can become in important human disorders.

4. Results

4.1. Molecular mechanisms of autophagy in mammals

4.1.1. Autophagic pathways

Proteins implicated in the mechanisms of autophagy are encoded by more than 30 genes, called *Atg* (autophagy-related genes), which were firstly identified in yeast, but they have also been described in other organisms as mammals[3,9]. It has been observed some similarities between autophagy in yeast and in mammals, however, not all the mechanisms implicated occur in the same way. Hereafter it is explained the mechanisms of autophagy in mammals. *Atg* proteins participate in the different steps of autophagy: induction or initiation, nucleation (formation of isolation membrane), membrane elongation, cargo recognition, sealing and fusion with lysosomes[10].

4.1.1.1. Autophagosome formation, cargo recognition and autophagosome sealing

The three first steps can be englobed in the process of autophagosome formation which requires 18 different *Atg* proteins (*AP-Atg proteins*). Autophagy is initiated by ULK1 (*Atg1)*, a serine-threonine kinase. During autophagy-inducing conditions ULK1 is dephosphorylated and the dissociation of the ULK complex (comprising ULK 1, FIP200, *Atg*101 and *Atg*13) from autophagy inhibitor mTORC1 complex occurs. After this dissociation, ULK1 becomes active and phosphorylates *Atg*13 and FIP200[10] (Fig.2).

The nucleation process starts when active ULK complex recruits Beclin1-Vps34 complex (comprising Beclin-1, Ambra 1, *Atg*14, Vps15 and Vps34, a class III phosphatidylinositol 3-kinase) to the site of autophagosome formation. ULK complex phosphorylates Ambra 1 and Beclin1. The phosphorylation of Ambra 1 produces its interaction with TRAF6 and stabilizes ULK1 through ubiquitination. The phosphorylation of Beclin-1 enhances the activity of the *Atg*L14 containing Vps34 complex. Active Vps34 synthesizes phosphatidylinositol-3-phosphate (PI3P) which binds DFCP1, an effector protein that promotes the initiation of double membrane vesicle nucleation (Fig.2), and another proteins such as WIPI 1, 2 and 4[10]. For example, WIPI-2 localizes on omegasomes (a cup-shaped protrusions from the ER formed mostly

of PI3P and implicated in the formation of the autophagosome), with DFCP1, and PI3P union suggests to act in the maturation of omegasomes into autophagosomes unveiling another origin for autophagosomes in mammals, the endoplasmic reticulum[11].

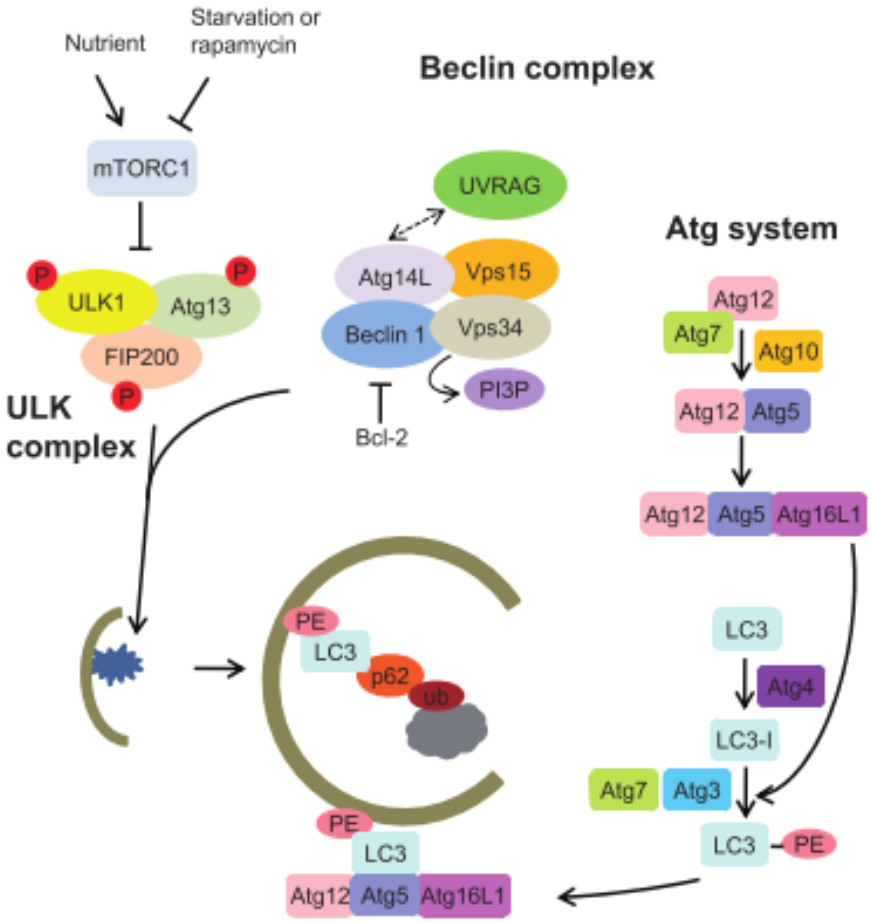

Figure 2. Induction of autophagy and autophagosome formation. During initiation ULK complex is activated and is dissociated from mTORC1 inhibiting complex. ULK complex recruits Beclin complex, in this one is included Vps34 which synthetises PI3P. Two conjugation systems are essential for autophagosome membrane elongation: the Atg12–Atg5 and the LC3–PE conjugation systems. Before autophagosome membrane closure, cargo recognition is produced by p62 protein family group. Conjugation of LC3 with *phosphatidylethanolamine* to form LC3II allows autophagosome closure. (Figure adapted from Ref. 12)

Vesicle elongation starts when the ULK1–Atg13–FIP200 and Beclin-1–class III PI3K complexes recruit two conjugation systems the *Atg*12–*Atg*5 and the *Atg*8–PE which promote membrane elongation (Fig. 2). The first one is mediated by two ligases *Atg*7 and *Atg* 10 and forms the *Atg*12–*Atg*5–*Atg*16 complex. In the second one, *Atg*4 acts to the soluble form LC3-I and produces LC3 (*Atg*8) which is conjugated by *Atg*7 and *Atg*3 to phosphatidylethanolamine forming LC3-II protein which allows the closure of autophagic vacuole. Both systems are essential for executing the conjugation cascades that mediate the conjugation and elongation of autophagosome membrane. LC3-II is

used as an autophagic marker[9] It is necessary to specify that before sealing (membrane closure) it is produced the recruitment of different cytoplasmic components such as proteins, organelles and pathogens as an autophagic cargo. This process is led by a mechanism that includes a protein family group with the protein p62 as the first member. This cargo recognition proteins present two distinctive domains, one of them interacts with different components of the autophagic machinery, as LC3, and the other one binds these proteins, organelles and pathogens. P62 recognises specific linkages of ubiquitin in the aggregated proteins or organelles, however it is thought that p62 union is not enough for protein aggregate removal by autophagy[3]. It has been observed that PI3P has also a role in selective cargo capture by the autophagosome by binding Alfy (Autophagy-linked FYVE protein), a protein involved in the selective degradation of ubiquitinated aggregates. Alfy interacts with *Atg*5 and p62 and PI3P, which is situated along the inner autophagosome membrane and directs the membrane building machinery to the site of p62 inclusion bodies providing a docking site for Alfy and selective engulfment of protein aggregates[11]. Organelles are also susceptible to selective recognition, for example, damaged mitochondria recognition is mediated by the autophagic adaptor p62/SQSTM[13].

4.1.1.2. Autophagosome maturation and fusion with lysosomes

Maturation of autophagosomes in major eukaryotes consists in the generation of amphisomes due to the fusion of autophagosomes with endosomes. In mammals the protein TECPR1 participates in this process as a tether factor, i.e. a protein implicated in vesicle trafficking which restrains autophagosomes and endosomes once they reach each other. TECPR1 needs to interact with the conjugated *Atg* 12-5 complex to bind PI3P and carry out its function as a PI3P effector[11].

Finally, fusion of amphisomes with lysosomes allows the degradation of the cargo material by lysosomal hydrolases and the obtaining of autophagic products in order to use them to synthetize new molecules or to obtain energy[4] (Fig. 3). Fusion step has not been well characterized in mammals, however, it has been found that in yeast a protein with a PI3P binding module (PpAtg24) is essential to mediate fusion between the vacuole and autophagosomes in yeast[4]. It has been observed that autophagosome

and lysosomal fusion is a temperature-depending process and requires ATP, GTP and an acidic pH inside the lysosome[14].

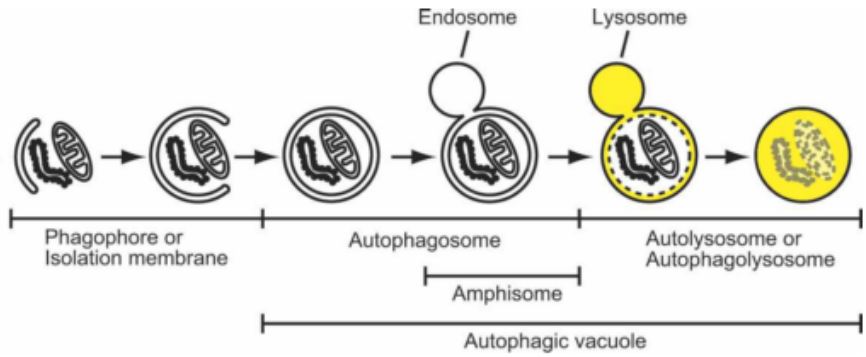

Figure 3. The process of autophagy in mammalian cells. Once autophagosome is formed, the maturation process takes place: amphisome is produced by the fusion of autophagosome with endosome. Later it is formed the autolysosome, when amphisome fusions with lysosome. Then internal material is degraded. All these vesicles are known as "the autophagic vacuole". (Figure adapted from Ref. 6)

4.1.2. Regulation of autophagy

4.1.2.1. The mTORC1 complex

Autophagy has a basal activity which is essential for the maintenance of intracellular homeostasis and that can be up-regulated or down-regulated by multiple signal–transduction pathways. The main complex implicated in autophagy inhibition and, consequently, in autophagy regulation is mTORC1. It is constituted by the association of the mammalian target of rapamicin (mTOR) with raptor and other modulatory proteins. The kinase mTOR is sensitive to changes of nutrients and energetic conditions inside the cell (Fig. 4). During normal conditions it interacts with autophagic inducers ULK1 and FIP200 phosphorylating them and avoiding its migration to the initiation site of autophagy, inhibiting this way autophagosome formation[3]. One of the pathways in which mTOR is implicated is the insulin-PI3K-PKB-mTOR pathway. In a general view, insulin trigger an intracellular response by binding its tyrosine kinase receptor that, after a serial of reactions, results into the formation of the active mTORC1 complex. Glucagon acts as opposed to insulin, it stimulates a pathway that ends in an inhibition of mTOR signaling. Indirectly, glucagon stimulates autophagy[11].

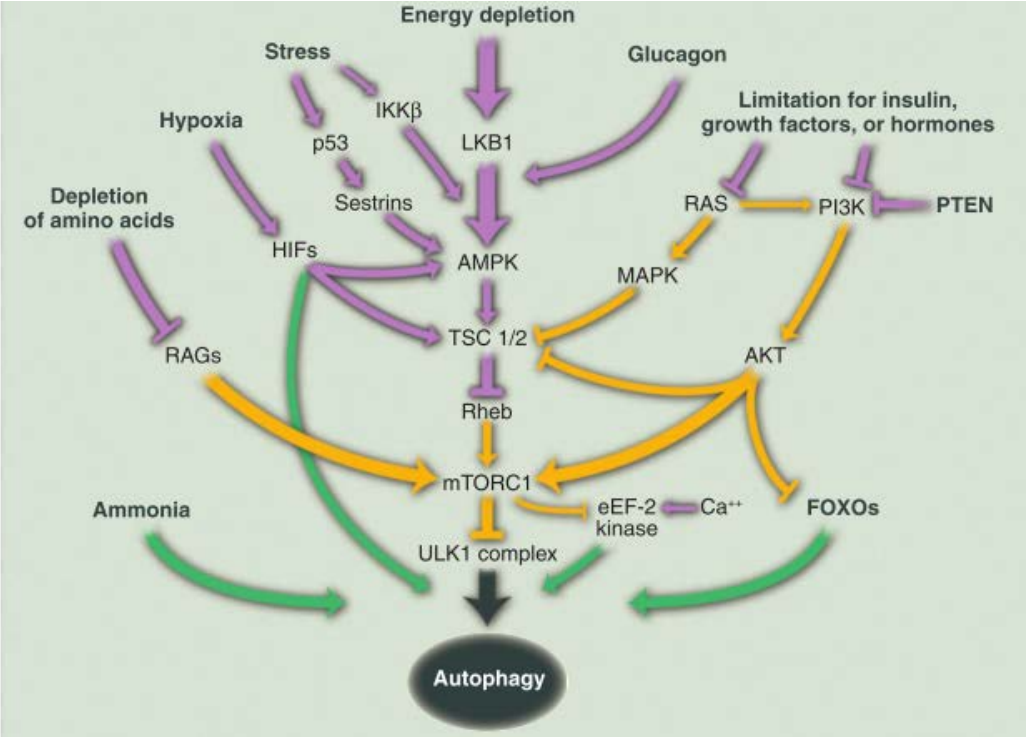

Figure 4. Regulation of autophagy, signaling pathways. The complex mTORC1 is the main system implicated in autophagy regulation. Almost all regulation pathways end in this complex. Its activation produces an inhibition of autophagy and it is inactivated by situations as amino acids depletion, hypoxia, stress, energy depletion or glucagon by different pathways and mechanisms. (Figure adapted from Ref. 15)

4.1.2.2. AMPK pathway and cellular energetic status

Cellular energetic status is one of the short-term elements of regulation that modulates autophagy by AMPK pathway. This mechanisms is also included inside the mTOR pathway, which is the most important element in autophagy regulation (Fig. 5).

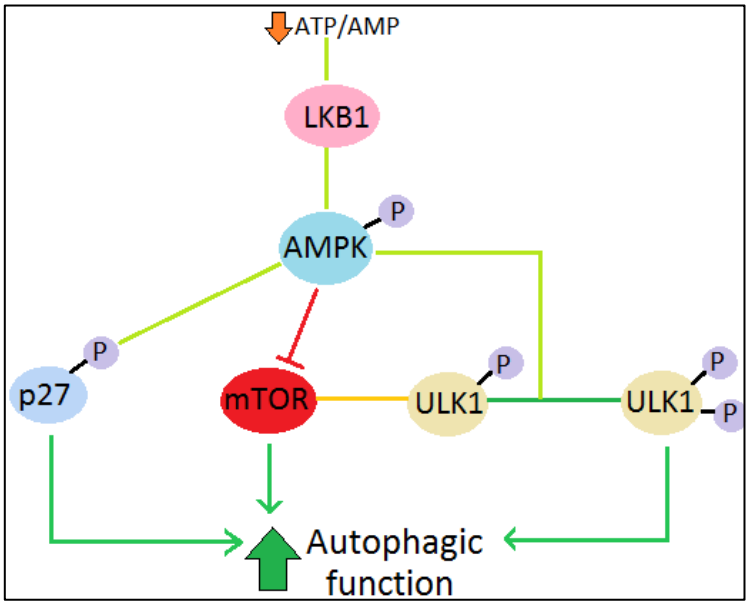

Figure 5. Cellular energetic status regulates autophagy.

Low ATP/AMP ratio is detected by LKB1 kinase, which activates AMPK (AMP-activated protein kinase) by phosphorylation. Then activated AMPK activates directly autophagy by phosphorylating p27 and also by phosphorylation of ULK1 (on serines 317 and 777) which dissociates from mTOR to initiate autophagy. AMPK also enhances autophagic function indirectly, it inhibits mTOR complex by direct phosphorylation of the Tuberous sclerosis complex 2 (TSC2) and Raptor and when mTOR is inhibited it does not phosphorylate ULK1 in 757 serine. In basal conditions, this serine is phosphorylated, preventing the union of ULK1 to AMPK[2].

4.1.2.3. The mTORC1 independent regulation.

There are also different forms of modulation of autophagy independent of mTORC1. During induction, it is important the participation of AKT and EGFR through phosphorylation of Beclin-1[10]. PI3P levels can also be modulated by Jumpy (MTMR14) and MTMR3, which are two PI3P phosphatases that regulate negatively autophagy[3, 11].

Autophagy can also be regulated by the extracellular signal-regulated kinases, ERK pathway. This pathway can increase or decrease autophagy, these effects are cell type dependent and stimuli specific[9].

4.1.3. Short-term and long-term regulation

There are some important elements in autophagy regulation and they are classified depending on the period of time that they need to occur. It is distinguished between a short-term and a long term regulation.

4.1.3.1. Short-term regulation

The elements implicated in a short term regulation are the Beclin-1-PI3K class III network (the binding of Beclin to class III PI3K is essential for the activation of autophagy), amino acids (inhibit autophagy by stimulation of mTOR signaling), cellular energy status (Low ATP/AMP ratio stimulates autophagy), oxidative stress (rise in ROS levels activate autophagy as a protective mechanism against damaged cellular components by ROS), endoplasmic reticulum stress (caused by unfolded protein response, UPR) and acetylation/de-acetylation (some of the proteins implicated in the

mechanism of autophagy are acetylated in nutrient-rich conditions and during starvation they are de-acetylated)[9].

4.1.3.2. Long-term regulation

The elements implicated in autophagic long-term regulation are FoxO proteins and the transcription factor EB, TFEB. FoxO proteins translocate into the nucleus, then they transactivate their target genes and induce autophagy; insulin produces FoxO sequestration in the cytoplasm, inhibiting indirectly autophagy. TFEB is not phosphorylated under starvation conditions and, then, it translocates to the nucleus to transactivate its target genes: proteins involved in cargo recognition, in vesicle formation and in substrate degradation)[9].

4.2. Nutrients implicated in autophagy regulation

4.2.1. Lipids and autophagy

It was thought that mobilization of lipids from lipid droplets was exclusively carried out by cytosolic or ER-associated lipases. Recently, an important role of autophagy has been described in lipid stores regulation and cellular lipid metabolism. This autophagic process receives the name of lipophagy[1,3].

Lipophagy allows triglycerides and cholesterol stored in lipid droplets to be taken up by autophagosomes and to be delivered into lysosomes for its degradation by acid hydrolases. This kind of autophagy is selective, it varies depending on cellular needs and external stimuli and has also the potential to regulate cellular energy homeostasis and lipid content. Starvation but also lipid supplementation increase the levels of lipophagy[1,3](Fig. 6). The mechanism of lipid droplet recognition remains unknown. However it has been observed an implication of LC3[1].

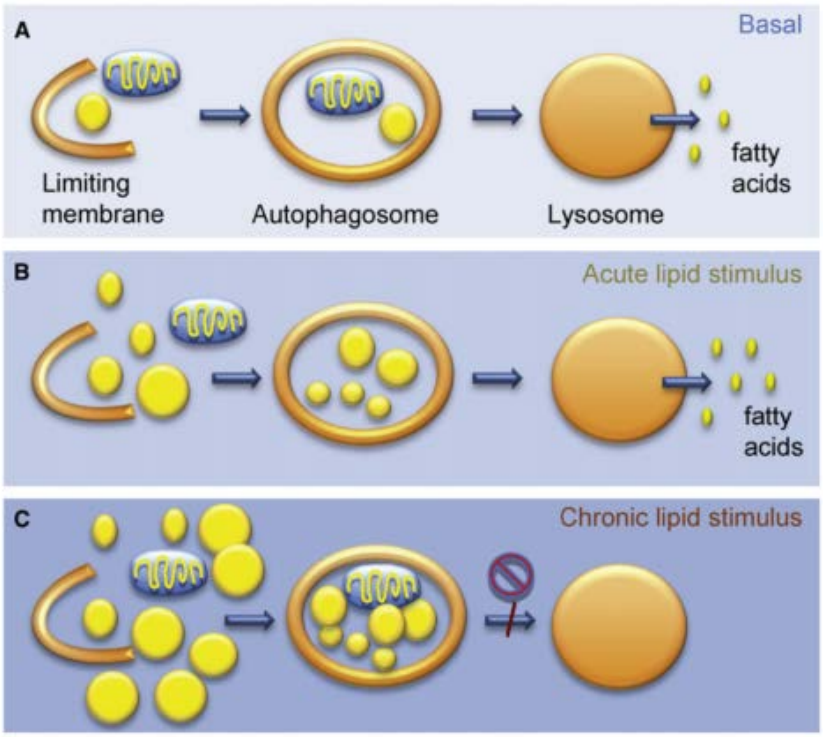

Figure 6. Starvation but also lipid supplementation enhances lipophagy. A. It exists a basal activity for lipophagy, where triglycerides are obtained by degradation of lipid droplets in lysosomes. B. An acute lipid stimulus enhances lipophagy activity preventing accumulation of lipids. C. Chronic lipid stimulation reduces lipophagy by decreasing autophagosome-lysosome fusion. It results in a high cellular lipid accumulation. (Figure adapted from Ref. 2)

4.2.1.1. Role of lipids in autophagic fusion step

Some studies showed a decrease in autophagic rates after chronical exposition to lipids (Fig. 6C). Studies *in vitro* and *in vivo* have revealed that changes in lipid composition reduce fusion of autophagic vesicles with lysosomes. The studies *in vitro* were reproduced by treating the cells with methyl-β-cyclodextrin, while studies in *vivo* were carried out by subjecting the animals to a high-fat-diet (HFD) challenge. In both cases, the final result was an increase in lipid content inside the cell, which may altered autophagy by inhibiting lysosome fusion with autophagy vesicles[14].

On this wise, while autophagy can mobilize lipid droplets and can degrade them through lipolysis, it can also be modulated by lipid content inside the cell. Moderate lipid stimuli increase autophagy. However, an acute exposure to high concentration of lipids or chronic lipid stimuli decrease not only lipid autophagy, but also long-lived proteins autophagy by inhibiting the fusion step[4,14].

4.2.2. Carbohydrates and autophagy

Glycogen can be degraded by acid hydrolases in lysosomes through autophagy, this process is called glycophagy and has been identified in liver and muscle. During post-natal starvation this process of glycophagy is essential for obtaining energy, and maintains a correct cell functioning and survival[2].

Carbohydrates can also have a regulatory effect on autophagy. Glucose deprivation enhances autophagy in general and an increase in glucose levels enhances autophagy of proteins by a mechanism of inhibition of mTOR by a phosphorylation pathway[2].

4.2.3. Proteins and autophagy

Autophagy is a key process in the metabolism of proteins. The principal purpose for protein breakdown is that to replenish the intracellular pool of amino acids required to maintain protein synthesis. During starvation, these amino acids act as substrates for gluconeogenesis and ketogenesis and serve the cell to get energy during starvation. Elimination of damaged proteins from the cytosol by autophagy maintains a correct cell functioning[2].

4.3. Food intake regulation

Autophagy is a cellular process which is activated, among others, in response to a low energy status (4.1.2.2) and, once activated, this process serves the cell to obtain energy from different cellular components during starvation. Through this mechanism, the cell can control its energy balance, acceding to a variety of sources of energy. In addition, autophagy is not only implicated in cellular energetic balance, but also in a global regulation of food intake in the whole organism. Some articles describe how hypothalamic autophagy could have a role in the control of food intake and energy balance[8,17,18,19].

4.3.1. Mechanisms of food intake regulation

4.3.1.1. Hypothalamus: master centre of food intake

Hypothalamus is a region of the brain formed by nuclei which are interconnected via axonal projections and are sensitive to changes in body energetic status. In response to these changes, the expression of neuromodulators and neurotransmitters is altered in the neurons that conform them and then, food intake and energy balance is modified. However, not all these nuclei participate in food intake regulation. Concretely, the arcuate nucleus (ARC) is considered the "*master hypothalamic centre of food intake*"[20]. Its situation (above the median eminence and close to the blood-brain-barrier) allows the neurons which conform it to monitor minute changes in circulating nutrients and hormones levels to get information about the energetic status of the organism. Peripheral nutritional signals are integrated by two functional antagonistic neuronal populations constituted respectively by POMC and AgRP neurons[8,20].

POMC neurons are located in the ventrolateral part of the arcuate nucleus and express the anorexigenic products of proopiomelanocortin (POMC) and the precursor of alpha-melanocyte stimulating hormone (α-MSH)[20]. The hormone α-MSH activates melanocortin 4 receptor on target neurons in the paraventricular nucleus reducing food intake[21]. The action of POMC neurons also promotes energy expenditure[8].

AgRP neurons are located in the ventromedial part of the arcuate nucleus and release two orexigenic neuropeptides: agouti-related protein (AgRP) and neuropeptide Y

(NPY). AgRP is an antagonist for the melanocortin 4 receptor and when it is secreted, it antagonizes α-MSH effect, resulting in a rising of food intake. AgRP neurons also focalize inhibiting projections to POMC by ϒ-Amino-butyric acid (GABA)[8,21].

AgRP and POMC neurons act in response to peripheral signals, such as insulin and leptin, and project to other secondary hypothalamic nuclei where depending on the signal received release neuropeptides that will modulate positively or negatively energy intake[20].

4.3.1.2. Leptin, the crucial anorexigenic peptide

Leptin is an anorexigenic polypeptide involved in hypothalamic feeding mechanisms. Its action consists in modulating the release of some neuropeptides and takes place over POMC and AgRP neurons. It is synthetized by adipose cells, providing a functional link between adipose tissue and hypothalamus. It is an indicator of energy status[20,22].

In the arcuate nucleus, leptin produces a depolarization of POMC neurons, increasing its neuronal activity, this fact produces a reduction of food intake and an increase in energy expenditure. Intracellular leptin signaling differs depending on two aspects, the type of signaling pathway (JAK/STAT or PI3K) and in which cell group (POMC or AgRP) the action of this peptide is produced (Fig. 7)[22].

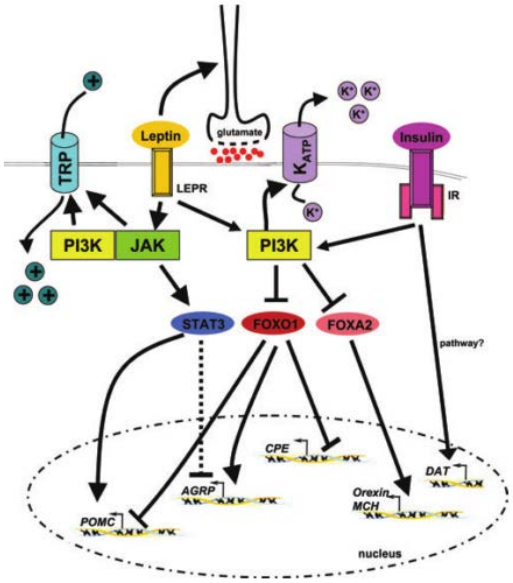

*Figure 7. Leptin intracellular signaling pathway. Leptin cascade includes the phosphorylation of STAT3 (signal transducer and activator of transcription) and its translocation into the nucleus to regulate the transcription of target genes, this way leptin increases POMC and inhibits AgRP transcription. Leptin also is able to stimulate activity of PI3K (phosphatydilinositol-3-kinase) which phosphorylates transcription factors FOXO1 and FOXA2. FOXO1 acts as an activator of AgRP transcription and as an inhibitor of CPE expression, an important enzyme in the obtaining of α-MSH (its final action its opposite to STAT3) (Adapted from Ref.*22)

4.3.1.3. Role of AMPK in the food intake regulation

AMPK is an important energy sensor produced, among others in the arcuate nucleus. Changes in its modulation affect directly in the physiological regulation of food intake. Activation of AMPK in the hypothalamus leads to an increase in food intake and also in body weight. Whereas repression of hypothalamic AMPK activity induces anorexia[20].

Changes in circulating nutrients and hormones produce effects which are associated to an activation or inhibition of hypothalamic AMPK. For example, hypoglycaemia activates AMPK in some hypothalamic nuclei, while glucose supresses AMPK activity. Changes in energetic status also modulate AMPK. For example, an increase in AMP, which indicates energy depletion, activates AMPK[8]. Leptin reduces AMPK activity in hypothalamus, supressing food intake. Ghrelin acts oppositely, increasing hypothalamic AMPK phosphorylation levels and activating it [20,23].

4.3.1.4. Role of fatty acids and CPT1 in the regulation of food intake

Activated AMPK inhibits acetyl CoA carboxylase (ACC). This results into a decrease in cellular production of malonyl CoA, the inhibitor of CPT1. When CPT1 is not inhibited, there is an increase in mitochondrial fatty acid availability and β-oxidation[8].

An increase in the hypothalamic levels of long chain fatty acids –CoAs (LCFA-CoA) is important for the physiological control of food intake. Studies unveiled that an inhibition of CPT1 and the subsequent elevation of long-chain fatty acids –CoA reduced the expression of NPY and AgRP and reduced feeding. Also, inhibition of fatty acid synthesis produces an accumulation of malonyl-CoA in the hypothalamus and reduces food intake and body weight[20].

4.3.1.5. Role of mTOR in food intake regulation

Leptin anorectic action can be blocked by the inhibition of PI3K (Fig. 7), which is an upstream target of mTOR (Fig. 4). This protein and a downstream target of mTOR action (SKG6) were found in rat hypothalamus, concretely in paraventricular and in the arcuate nuclei. After analysing the two neuronal populations, phosphorylated mTOR

and SKG6 were found in the 90% of AgRP neuron. However, these elements were found in only a 45% of POMC neurons[24].

This protein kinase has a role in hypothalamic response to changes in energy status, it is thought to serve as an ATP sensor and has an important function detecting changes in energy status and transducing this signal. It is also sensitive to levels of leucine. The induced increase in hypothalamic mTOR signalling pathway, by injecting leucine beside the arcuate, decreases food intake and body weight. It has been recently described the importance of mTOR in leptin intracellular signaling. It has been observed that leptin produces a rise in mTOR hypothalamic activity, which phosphorylates SKG6 and inhibits AgRP expression, reducing food intake and body weight[25]. Also, an inhibition of mTOR signaling produces a resistance to the anorectic effects of leptin. Thus, leptin's action results from an intracellular activation of mTOR and its effect is mTOR depending[24,25].

4.4. Role of autophagy in food intake regulation

After the revision of information in relation to autophagy and food intake regulation, it can be observed that there are some common elements, concretely common proteins which are implicated in both mechanisms: autophagy and food intake regulation in hypothalamus. Consequently, some experts decided to study deeply how autophagy was induced in hypothalamus, if autophagy's role in hypothalamus was related to food intake regulation and in an affirmative case, which were the mechanisms of autophagy implicated in food intake regulation in neuronal populations of the arcuate nucleus.

4.4.1. Autophagy in hypothalamic cells is induced by starvation

Both, constitutive and adaptive autophagy, occur in the central nervous system[18]. Basal levels of autophagy are important to maintain the functionality of hypothalamic neurons. However, it was necessary to study which stimulus induced hypothalamic autophagy to study its possible role in this zone. Two different studies in hypothalamic GT1-7 cells (*in vitro*) and in C57BL/6 mice (*in vivo*) demonstrated that autophagy was induced by starvation. Although previous studies indicated that hypothalamic autophagy was starvation resistant or constitutively active, the ability of hypothalamic neurons to upregulate autophagy after starvation was demonstrated. There is also an evidence in the opposite fact, after nutrient resupplementation of GT1-7 cells, it was observed a decrease in autophagy levels by blocking AMPK signaling and by an activation of mTOR[8,17].

4.4.2. Autophagy in AgRP and POMC neurons

It was observed that hypothalamic free fatty acid (FFA) levels inside AgRP neurons increased during starvation. It was proposed that after the production of FFA by peripheral starvation-induced autophagy, they were incorporated by AgRP cells. Once FFA were in AgRP neurons cytosol, they activated autophagy which mobilized neuronal lipid stores and increased endogenous FFA[8].

There is a link between fatty acids derived from autophagy and AgRP expression, an increase in these fatty acids ended in an increase of AgRP levels. A selective deletion of

autophagy in AgRP neurons by deleting *Atg*7 gene in mice reduced body weight and AgRP levels. It was also observed that inhibition of autophagy in AgRP neurons increased hypothalamic levels of POMC precursors, and this fact could be the reason for the body weight reduction in these mice[8,17] (Fig. 8 and 9).

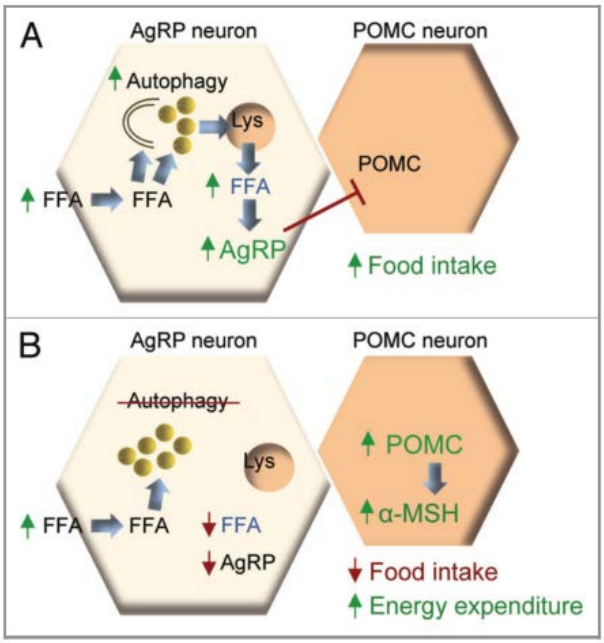

*Figure 8. Role of autophagy in AgRP neurons in the control of food intake. A. Starvation increases hypothalamic FFA uptake which activate autophagy. New fatty acids are produced by autophagy, which originate an augment in AgRP levels and, consequently, in food intake. B. After inhibiting autophagy, starvation and the subsequent FFA uptake by the neuron are not capable to produce new FFA neither AgRP. AgRP neurons do not inhibit POMC neurons, which produce POMC and α-MSH, reducing food intake and increasing energy expenditure. (Adapted from Ref.*8).

After studying the role of autophagy in AgRP neurons, the next step was to find out what was its role in POMC neurons. Conditional KO mice were generated: they didn't synthetize the autophagic protein *Atg 7* in POMC neurons and autophagy was not functional in this cells. These mice presented a high body weight that was due to an increase in food intake and in body fat (adiposity)[26]. Loss of autophagy in POMC neurons increased hypothalamic levels of prePOMC and ACTH (its cleavage product) and it was inhibited the generation of α-MSH. They concluded that autophagy modulated the production of α-MSH from prePOMC protein in POMC neurons, and that was the reason why there was an increase in food intake in these mice. Autophagy in POMC neurons has also a role in a correct peripheral lipolysis. Thus, dysfunctional autophagy in POMC cells impaired peripheral lipid mobilization, fact that produced an increase in body fat mass. Then, POMC neurons control sympathetic outflow to different organs which induces lipolysis[18,27] (Fig. 9).

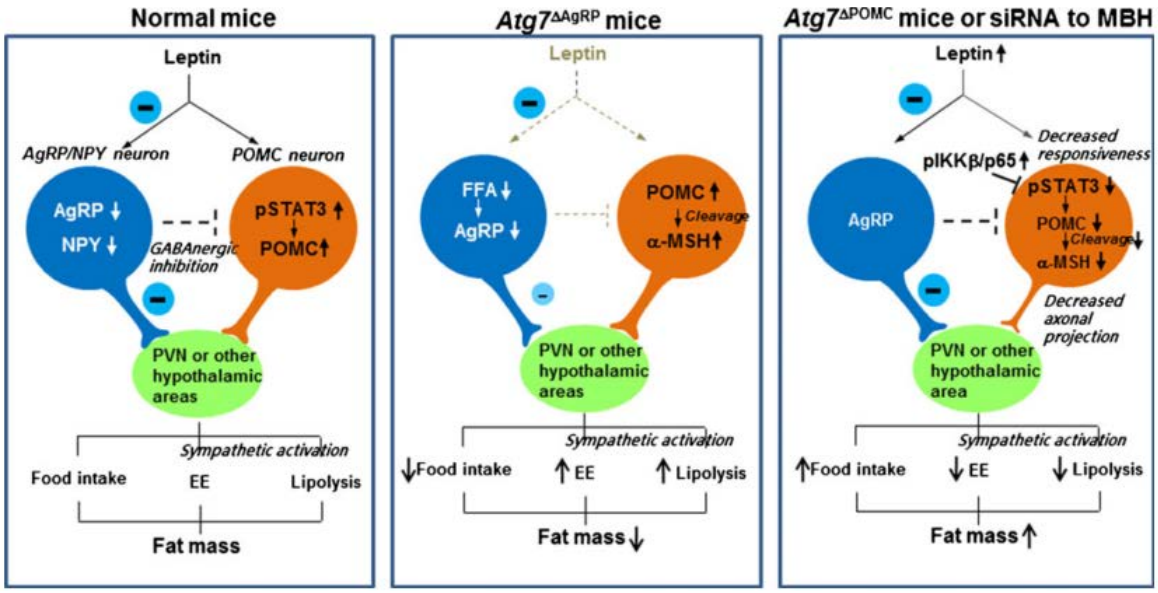

Figure 9. Comparison between normal mice, AgRP and POMC autophagy-deficient mice in relation to food intake. In normal mice, AgRP and POMC neurons regulate food intake, energy expenditure and lipolysis normally. In AgRP autophagy-deficient mice, there is a decrease in AgRP production (mediated by autophagy) and a deficient inhibition of POMC neurons, which results in α-MSH increase, a reduction of food intake and a rising in energy expenditure and lipolysis. Contrarily, in POMC autophagy-deficient mice, there is a decrease in POMC cleavage into α-MSH and consequently, an increase in food intake and a reduction in energy expenditure and lipolysis. (Adapted from 18)

4.4.3. Autophagy as a mechanism involved in the leptin effect on food intake

Leptin is a crucial hormone in the control of food intake (4.3.1.2). After some evidences that showed that leptin promoted autophagy[27], it was thought that leptin regulates food intake through a mechanism of autophagy. To study this hypothesis, HFD fed mice with deficient autophagy in POMC neurons (by deleting *Atg7* gene in these neurons) have an impaired response to leptin compared to wild type mice. It was observed that the response to α-MSH intracerebroventricular administration was not affected, thus, autophagy was not implicated in two second order neurons response. Also, STAT3 (the major signaling pathway for the central metabolic effects of leptin) was reduced in these mice's POMC neurons. Consequently, it was concluded that this damaged response to leptin was due to defects in the signal transduction of this hormone. It is not known which the mechanisms of leptin resistance are. However, there are some options that have been proposed: it could be implicated ER stress, inflammatory process related to autophagy, deficiency in intracellular ATP and the accumulation of ubiquitinated proteins[18,19].

4.4.4. Controversial role of the autophagic *Atg* proteins in POMC neurons

In previous experiments, autophagic function in AgRP and POMC neurons, were studied by deleting *Atg7* gene in both and it was accepted that *Atg7* had an exclusive role in autophagy and not in other mechanisms in these neurons. For these reason, *Atg7* deletion was translated into autophagy deficiency in these cells and the results were interpreted in one concrete direction: autophagy had a role in the control of food intake and energy expenditure through a mechanism related with leptin.

A research group decided to investigate what happened if instead of deleting *Atg7* gene in POMC neurons delated *Atg12* and *Atg5* in the same neurons. In both cases, there was autophagy ablation but the results were surprising. When mice were fed with a normal diet, genetic deletion of *Atg12* and *Atg5* in POMC neurons did not promote weight gain or adiposity. They also discovered that *Atg12* followed the pattern of *Atg7* deletion in HFD fed mice, but *Atg5* deletion had no effects neither in food intake nor energy expenditure in HFD fed mice. Thus, they concluded that neither the absence of the *Atg12-Atg5* complex in POMC neurons nor the lack of autophagy are not responsible for increased body weight gain on HFD. They discussed the possibility of a non-autophagic alternative function for *Atg12* (and also *Atg7*) in POMC neurons that prevent excessive weight gain in animals upon HFD challenged[28].

The fact that non-autophagic functions have been described for some *Atg* genes[29,30], lead to think that *Atg7* and *Atg12* proteins in POMC may influence in weight gain but not through a mechanism of autophagy.

4.5. Human disorders related to a defective autophagy

Although it is not still clear if autophagy has a role in hypothalamus in relation with food intake, some human disorders have been related to an alteration of autophagy in this region. In relation to hypothalamic autophagy, its possible role in the development of some diseases such as obesity or metabolic syndrome has been observed.

4.5.1. Disorders related to autophagy in relation to food intake regulation

Studies unveiled that chronic lipid stimulation reduces lipophagy by decreasing autophagosome-lysosome fusion and that resulted in a high cellular lipid accumulation (4.2.1). This discovery lead to suppose that diet, specially that one with high content in lipids, could influence in the regulation of autophagy.

After studying the effect of diet induced obesity in hypothalamic neurons, it can be deduced that under conditions of chronic excess of fatty acids in the diet, neuronal autophagy is compromised. Impaired autophagy may lead to inflammation that could end up in neuronal apoptosis and consequently in loss of hypothalamic control of appetite, food intake and energy balance. Thus, loss of hypothalamic neurons involved in regulating energy homeostasis is one of the reasons that food intake is difficult to control on chronic obesity[31].

4.5.2. Some other disorders derived from an alteration of autophagy in the CNS

Furthermore, there are other human disorders in which has been observed a deficient autophagy in the central nervous system (CNS). Among all its functions, autophagy is also a homeostatic process which participates in cellular quality control, removing from the cytosol all those components which are dangerous to the cell and can compromise cellular viability.

Autophagy is essential for the survival of neural cells and a failure of autophagy in the central nervous system is implicated in pathogenesis of some neurodegenerative

diseases. The specific suppression of autophagy in the CNS results in the accumulation of abnormal proteins, because neurons are incapable of removing aggregates from its cytosol. This fact causes general cellular dysfunction, and neurodegeneration. Contrarily, activation of autophagy in these cells reduces accumulation of damaged proteins and organelles and reduces cell death[32]. Lack of autophagy has been associated to Huntington disease, Parkinson disease and amyotrophic lateral sclerosis[1,6].

The dysfunction of two forms of autophagy, mainly CMA but also macroautophagy, influences in the pathogenesis of Parkinson disease. CMA is the autophagic pathway which is mainly implicated in the elimination of α-synuclein (which is the major constituent of Lewy bodies, the protein inclusions in affected brain regions in Parkinson disease). When α-synuclein is mutated, it blocks CMA activity and the degradation of other CMA substrates. The overexpression of α-synuclein reduces autophagosome formation in macroautophagy and the loss of function of PINK1 and Parkin (two proteins associated with Parkinson disease) causes defects in the degradation of mitochondria through mitophagy (macroautophagy pathway of mitochondria) in neurons, mitochondria cannot be eliminated and this causes apoptosis and neurodegeneration[32].

Huntington disease is characterized by an expansion of the CAG trinucleotide repeat encoding a polyglutamine tract in the Huntingtin protein. This aberrant protein causes a loss of its normal function and gain of toxic function. Normal Huntingtin is degraded by UPS (Ubiquitin-proteasome system). However, abnormal Huntingtin is degraded preferentially by autophagy, but the toxic function of the polyglutamine tract alters autophagy in the cargo recognition, in vesicular trafficking and fusion and impairs autophagy in the neurons in Huntington disease[32].

5. Discussion

5.1. Autophagy and food intake regulation

Autophagy is a catabolic process of degradation of intracellular components in lysosomes. It participates in several cellular mechanisms such as quality control, energy balance or defence among others. Its basic role depends on the tissue; however, its molecular mechanisms are equal for all cell types[3].

Activation of autophagy is a critical response to nutritional depletion[8]. During starvation, autophagy is induced in order to provide energy and nutrients to the cell and thus helps the cell to maintain its energetic balance[7]. This fact brought to some investigators to think that autophagy might also be important for the maintenance of whole body metabolism in the physiological state, being implicated in the regulation of food intake[18]. After studying some mechanisms implicated in food intake regulation in the hypothalamus, it was observed that signaling cascades that regulate autophagy also regulate food intake[8]. It was also observed that hypothalamic fatty acid metabolism was implicated in food intake regulation[20] and Sigh's research group found an implication of autophagy in fatty acid metabolism (lipophagy)[33]. All this evidences brought investigators to study deeply what was the role of autophagy in the hypothalamus.

Firstly, Kaushik's group found that autophagy was nutritional regulated in GT1-7 hypothalamic cells and they proposed that peripheral FFA induced autophagy in hypothalamus and this mechanism provided neuronal availability of endogenous FFA which were implicated in the release of AgRP (the orexigenic peptide) and incremented food intake. In order to study how hypothalamic autophagy influenced in the whole organism, they also created mice that lacked the autophagy gene *Atg7* specifically within the AgRP neurons to inhibit autophagy. This inhibition reduced significantly body weight. They concluded that loss of autophagy in AgRP neurons decreased the release of AgRP and that produced a decrease in body weight[17].

Secondly, role of autophagy was studied in POMC neurons. Quan's group generated mice with POMC neuron-specific *Atg7* deletion. This deletion produced autophagy

deficient POMC neurons. Food intake was significantly higher in these mice. They concluded that Autophagy may be implicated in leptin molecular mechanism of action and that was necessary for POMC cleavage into α-MSH and reduce food intake[19].

However, all these results were derived from study de deletion of *Atg7* gene in AgRP and POMC neurons. Dysregulation of food intake was exclusively attributed to a loss of autophagy but not to the loss of an alternative function of *Atg7* (these alternative functions exist for almost all *Atg* proteins[29]). It was necessary to study deeply what was the implication of autophagy in the regulation of food intake, by deleting other *Atg* proteins. For these reason Malhotra's group generated mice with POMC neuron-specific *Atg12* and *Atg5* deletion. Hypothalamic autophagy in these mice was deficient, *Atg12* deficient mice incremented food intake but it did not happen in *Atg5* deficient mice[28].

It is not possible to define if autophagy has a role in the regulation of food intake with the evidence found until now. These results do not demonstrate neither autophagy has a role in the mechanisms of food intake regulation nor it has not. Contrarily, it is possible to affirm that *Atg7* and *Atg12* proteins have a kind of role in the mechanisms of food intake regulation. It would be necessary to study the roles of the deletion of other *Atg* proteins in AgRP and POMC neurons in order to find if they have new non-autophagic functions and what is its individual role in the control of food intake. It would be also necessary to find a way to inhibit autophagy without altering *Atg* proteins in AgRP and POMC neurons, and this way it could be possible to obtain more specific results about the implication of autophagy in the regulation of food intake.

5.2. Human disorders related to a defective autophagy

It is necessary to comment that even though autophagy could not be related to food intake regulation, there are some human disorders related to the lack of a correct autophagy in the CNS.

Chronic HFD causes obesity and autophagy impairment in hypothalamus, which could lead to inflammation and apoptosis[31]. Thus, if autophagy had a real role in food intake regulation, both incidents (autophagic role and neuronal death due to inflammation)

could conduce to loss of hypothalamic control of appetite, food intake and energy balance in these organisms.

Autophagy has a clearer role in the pathogenesis of other neuronal disorders: Parkinson's and Huntington's diseases[32]. This role is clearer because is related with quality control function of autophagy, which has been well-defined.

6. Conclusion

1. Autophagy is a cellular process with different roles and behaviours depending on the tissues. However, the autophagic pathway from induction to fusion and regulation are equal.
2. Principal macronutrients are implicated in this cellular process. Lipids, carbohydrates (glycogen) and proteins can be degraded by the autophagic pathway. Lipids and carbohydrates have a regulatory effect on autophagy. Acute lipid stimulus and glucose deprivation enhance autophagy, while a chronic exposure to lipids produces a decrease in autophagic rates.
3. The implication of autophagy in the food intake regulation is under debate. While it could participate in the release of AgRP (the orexigenic peptide) and in leptin satiety action on POMC neurons, some Atg proteins involved in autophagy do not have any role in the regulation of food intake. Further studies are required for understanding the real role of different *Atg* proteins and autophagy in hypothalamic control of food intake.
4. Some human disorders are related to a lack of a correct autophagy in the central nervous system. In diet-induced obesity there is an impairment in hypothalamic autophagy, which could lead to the loss of hypothalamic control of appetite and food intake. Other CNS disorders where autophagy pathway is altered are Parkinson's and Huntington's diseases.

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