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Neuronal autosis: the self-destructive side of autophagy involved in hypoxic-ischemic neuronal death

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The challenge of protecting the brain resides in the unique characteristics of neurons, as they are postmitotic, long-lived, excitable, and polarized cells with long and fragile axons and dendrites. The complexity of the multiple potential cell death pathways further complicates this issue. In addition, the immature brain is prone to a "cell death continuum," which involves intricate molecular interconnections between cell death processes. This makes finding safe and effective neuroprotective strategies to prevent damage to the developing brain a significant challenge in neonatology. The only approved treatment for term newborns with hypoxic-ischemic encephalopathy (HIE) is therapeutic hypothermia. However, access to this treatment and its effectiveness is limited to a few cases. Research is focused on developing new neuroprotective agents that can be combined with hypothermia to improve its therapeutic window and outcome.

We and others have demonstrated that neuronal autophagy is enhanced after hypoxia-ischemia and/or excitotoxicity in primary neuronal cultures (Ginet et al., 2014b; Descloux et al., 2018), rodent pups (Liu et al., 2013; Ginet et al., 2014b; Xie et al., 2016; Descloux et al., 2018), and brain sections of human newborns with severe HIE (Ginet et al., 2014a; Xie et al., 2016). Genetic downregulation of BECLIN1 (BECN1) (Ginet et al., 2014b), neuron-specific knockout of autophagy-related genes (atg) 7 (Xie et al., 2016) or pharmacological autophagy inhibition (Descloux et al., 2018) provides neuroprotection in these different preclinical models of perinatal brain injuries. A better understanding of the connections between enhanced autophagy and neuronal death may open the door to developing new therapeutic options for HIE.

Indeed, the specific process by which autophagy, an important physiological and adaptive mechanism, is converted into a deleterious one remains unknown. The role of autophagy in cell death has long been controversial, particularly in pathogenesis rather than development. This emphasizes the critical need for further research to unravel the complexities of autophagy and its potential involvement in cell death (Clarke and Puyal, 2012). Enhanced autophagy, characterized by an unusually high presence of autophagosomes and autolysosomes, has traditionally been viewed as the primary characteristic of autophagic cell death—an additional form of cell death alongside apoptosis and necrosis. In addition to these predominant types of cell death, various forms of programmed cell death have been identified and classified based not only on ultrastructural morphological features but also on molecular, pharmacological, and functional properties (Klionsky et al., 2021; Park et al., 2023). Among these diverse forms of programmed cell death, autosis has been recently described as a distinct type of autophagic cell death with specific morphological and molecular features. This discovery provides compelling evidence for the involvement of autophagy as a direct initiator of cell death.

The morphological definition of autosis includes the presence of numerous autophagosomes, autolysosomes, and empty vacuoles in the cytoplasm during the initial phase, such as during "classical" autophagic cell death. However, during the second phase, a focal swelling of the perinuclear space (PNS) occurs, and this swelling is considered the primary morphological signature of autosis (Liu et al., 2013; **Figure 1**). In the late stage, a reduced number of autophagosomes/autolysosomes is accompanied by necrotic-like features such as organelle swelling and loss of plasma membrane integrity (as observed during "classical" necrosis). The focal swelling of PNS facing a concave nucleus at this stage serves as the main criterion for differentiating autosis from necrosis. This raises the question of whether autosis may have been underestimated or misinterpreted as necrotic cell death in certain pathological conditions, and whether all reported forms of autophagic cell death are instances of autosis or if autosis represents a distinct type of autophagic cell death. The specific morphological and molecular characteristics of autosis suggest that there are now tools available to systematically investigate these possibilities.

Since its initial description in 2013 in HeLa cells exposed to the cell-permeable autophagy-inducing peptide Tat-BECN1, autosis has garnered increasing interest, and some progress has been made in understanding its molecular mechanisms. Autosis relies not only on canonical atg genes such as atg7, becn1, and atg5, but also specifically on the α subunit of the Na^+K^+-ATPase (ATP1a) (**Figure 2**). It has been demonstrated that cardiac glycosides, which act as ATP1a ligands, could be used as specific pharmacological inhibitors of autosis at low doses (Liu et al., 2013). The protective nanomolar doses of cardiac glycosides, which do not affect the ion pumping function of ATP1a, suggest that ATP1a mainly induces autosis through its signal transducer function (Škubník et al., 2021).

In this context, research has demonstrated that in autosis conditions, BECN1 interacts with ATP1a in various cell types (Liu et al., 2013; Fernández Á et al., 2020; Depierre et al., 2024). As BECN1 (an ortholog of yeast ATG6) forms different protein complexes throughout the autophagic process and is involved in both autophagy induction and maturation, it has been proposed that the BECN1/ATP1a interaction may play a crucial role in autosis. In starved primary cardiomyocytes, it has been proposed that the degradation process of autophagy plays a less significant role in autosis compared with the initial phase of autophagosome formation (Nah et al., 2020). The massive production of autophagosomes during autosis may lead to the depletion of membrane components from the plasma membrane and intracellular organelles, resulting in the dysfunction of essential organelles such as mitochondria or endoplasmic reticulum. Notably, the interaction between BECN1 and the α1 subunit of ATP1 (ATP1a1) has been observed at the plasma membrane and some of these intracellular compartments, including mitochondria, endoplasmic reticulum, early endosomes, and nuclear membrane.

Starvation or hypoxia/ischemia with reperfusion are well-known conditions that boost autophagy and have been identified as the primary (physiological) triggers for inducing autosis. The occurrence of autosis has been documented in various proliferating and/or cancer cells (such as cardiomyocytes, HeLa cells, and Panc-1 cells), in different rodent organs (liver, heart, kidney, and brain), and even in human livers of patients suffering from anorexia nervosa (**Figure 2**). Autosis has also been observed in dying neurons in a rat preclinical model of HIE, where the cardiac glycoside neriifolin demonstrates potent neuroprotective effects (Liu et al., 2013; Ginet et al., 2014a).

In a recent study (Depierre et al., 2024), we conducted a detailed analysis of neuronal autosis in primary cortical neurons using Tat-BECN1 and a more physiologically relevant stimulus combining hypoxia and excitotoxicity that mimics hypoxic-ischemic insult. We found that neuronal autosis occurs independently of apoptotic and necroptotic mechanisms, as observed in other cell types. Notably, we also demonstrated that neuronal autosis is distinct from ferroptotic mechanisms, indicating that these cell death pathways are not interconnected. Furthermore, we identified that neuronal autosis is reliant on the neuronal-specific subunit α3 of ATP1a (ATP1a3), rather than the ubiquitous ATP1a1 observed in other cell types. Our research revealed that in conditions leading to autosis, BECN1 interacts with ATP1a3 in neurons (Liu et al., 2013; Fernández et al., 2020; Depierre et al., 2024). This interaction was observed in primary cortical neurons in culture, as well as in a rat preclinical model of HIE and human newborn brains with severe HIE (Depierre et al., 2024). These findings strengthen the notion that autosis may play a significant role in hypoxia-ischemia-induced neuronal death, highlighting the potential for targeting neuronal autosis for neuroprotection in hypoxic-ischemic conditions.

While digoxin is currently used to treat cardiac pathologies in pediatric patients, cardiac glycosides have a narrow therapeutic range and significant side effects, limiting their clinical application for HIE. Research aimed at identifying the downstream ATP1a3 signaling interactome involved in autosis will enable the specific targeting of neurons (as ATP1a3 is only expressed in neurons), thus avoiding potential side effects of autophagy inhibition in glial cells (including developing oligodendrocytes and reactive astrocytes). This research holds promise for proposing new therapeutic options for HIE.

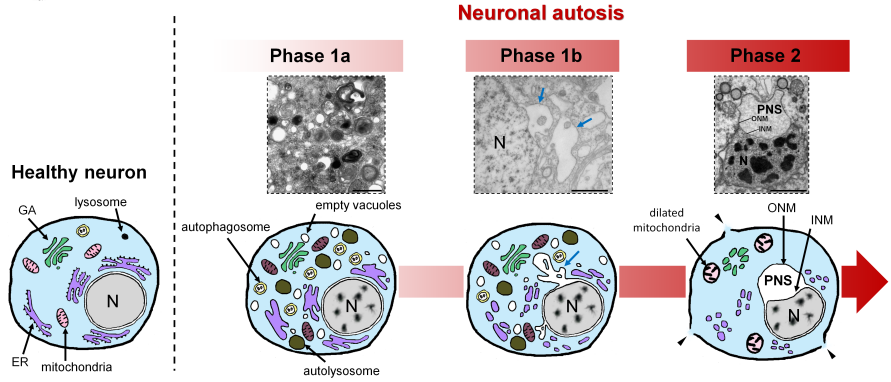

Figure 1 | **Morphological features of neuronal autosis.**
Neuronal autosis is divided into two main phases as illustrated in the different schemes and representative electron micrographs of CA3 hippocampal dying neurons after hypoxic-ischemic injury in a rat preclinical perinatal model. During the first stage of phase 1 (phase 1a), numerous autophagosomes, autolysosomes, and empty vacuoles are observed. The PNS appears normal, but chromatin condensation begins without forming well-defined clumps as seen in apoptosis. Some mitochondria are electron-dense, and the ER appears dilated and fragmented. During phase 1b, the nucleus starts to convolute, and some restricted regions of the PNS (blue arrows) display swelling and contain membranous structures with cytosol-resembling content. During the second phase autophagosomes and autolysosomes became scarce, and dying neurons have a necrotic-like morphology. Almost all organelles are swollen and fragmented, and the plasma membrane is ruptured (arrowhead). At this stage, the main morphological criterion to distinguish autosis from necrosis is the presence of a focal ballooning of the PNS facing a concave nucleus, which is the morphological signature of autosis. Scale bars: 1 µm. Unpublished data. ER: Endoplasmic reticulum; GA: Golgi apparatus; ONM: outer nuclear membrane; INM: inner nuclear membrane; N: nucleus; PNS: perinuclear space.

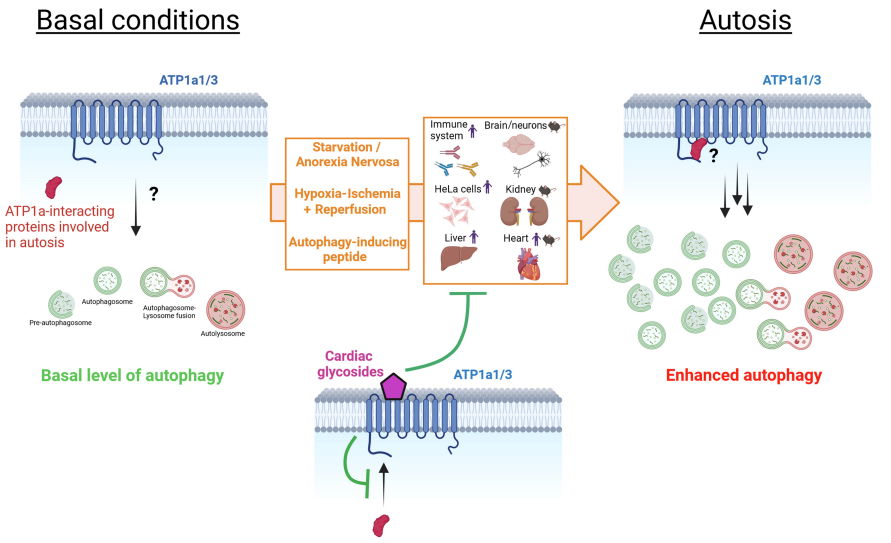

Figure 2 | **Molecular mechanisms involved in autosis.**
In basal conditions, healthy cells display a basal level of autophagy, represented by a small amount of autophagosomes and autolysosomes. The main conditions reported to induce autosis include treatment with autophagy-inducing peptides (such as Tat-BECN1), starvation, and hypoxia-ischemia followed by reperfusion. Autosis has been reported to occur in various proliferating cells, including cardiomyocytes, HeLa cells, and Panc-1 cells, as well as in rat primary cortical neurons and different rodent organs such as the liver, heart, kidney, and brain. It has also been observed in human livers of patients suffering from anorexia nervosa. In such conditions, autosis is dependent on ATP1a1, except in neurons where autosis is ATP1a3-dependent. The involvement and role of ATP1a in (basal) autophagy remains to be investigated (question marks). Cardiac glycosides, which are direct ligands of the ATP1a, are potent autosis inhibitors. ATP1a is proposed to act as a signal transducer by recruiting proteins involved in autosis induction. The downstream ATP1a interactome involved in autosis needs further characterization. It has been described that when autosis is induced, BECN1 (mammalian homolog of ATG6) interacts with ATP1a1/3. Cardiac glycosides can prevent ATP1a/BECN1 interaction and autosis. The interacting region of BECN1 on ATP1a has not yet been identified (question marks). Created with BioRender.com.

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