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Excitotoxicity and Wallerian degeneration as a processes related to cell death in nervous system

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ABSTRACT

Cell death is one of the processes that are currently extensively studied. Beside the commonly used terminology regarding cell death, i.e. apoptosis, autophagy, necrosis, and cornification, in recent years there has been a growing number of additional definitions of this process, such as mitotic catastrophe, anoikis, entosis, paraptosis, pyroptosis, pyronecrosis, excitotoxicity, and Wallerian degeneration, which are described as atypical by the 2009 Nomenclature Committee on Cell Death. The recent report of that Committee significantly alter the classification and nomenclature of the cell death processes, in which excitotoxicity and Wallerian degeneration have not been taken into account. Thus the present review describes excitotoxicity, and Wallerian degeneration, as two processes associated to cell death phenomena characteristic for nervous system.

Excitotoxicity is a neuronal death caused by excessive, or prolonged activation of receptors for the excitatory amino acids. Depending on the intensity of the initiating stimulus, the excitotoxicity may overlap with other types of cell death such as apoptosis and necrosis.

Wallerian degeneration is a process that results when a nerve fiber is cut or crushed, in which the part of the axon separated from the neuron's cell body degenerates distal to the injury. Wallerian degeneration is not a typical cell death mechanism, since neurons undergoing this process remain alive.

Key words

Cell death • Excitotoxicity • Wallerian degeneration

Introduction

For many years cell death has been a subject of extensive studies of scientist from all over the world. The processes of cell death occur in healthy, as well as pathologically changed tissues of the organism. Until recently it was considered the existence of four basic types of cell death: apoptosis, autophagy, necrosis, and cornification (keratinization), based on the recommendation of the NCCD (The Nomenclature Committee on Cell Death, 2009). Apoptosis is a type of programmed cell death mediated by caspases, during which some characteristic features can be observed, such as: reduction of cellular volume, condensation and fragmentation of chromatin, formation of apoptotic bodies, and their phagocytosis by neighboring phagocytes, but without induction of inflammation processes (Kroemer et al., 2009). Necrosis is cell death accompanied with local inflammation and is characterized by a gain in cell volume (oncosis), swelling of organelles, plasma membrane rupture and subsequent release of the cellular contents into the intracellular space (Kroemer et al., 2009). Autophagy, in terms of cell death process, is defined as a type of cell death proceeding without chromatin condensation, but

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with an intense vacuolization of the cytoplasm, and autophagosome formation (Kroemer et al., 2009). Cornification is a very specific type of programmed cell death, which leads to formation of corneocytes – dead keratinocytes containing structural proteins, fatty acids, and ceramides (Kroemer et al., 2009). Moreover, mentioned guidelines of the NCCD (Kroemer et al., 2009), give tentative definitions of 8 atypical cell death modalities: mitotic catastrophe, anoikis, entosis, paraptosis, pyroptosis, pyronecrosis, excitotoxicity, and Wallerian degeneration. Mitotic catastrophe is a form of cell death occurring during, or shortly after abnormal mitosis, which is accompanied by multi-nucleation and/or formation of micronuclei from the chromosomes and their fragments that have not been evenly distributed between daughter nuclei during mitosis (Green et al., 2009). Anoikis and entosis belong to atypical cell death mechanisms connected with interruption or loss of the proper cell-cell, and cell-ECM (extracellular matrix) adhesions (Gilmore, 2005; White, 2007). The term paraptosis was introduced to describe a programmed cell death (PCD) distinct from apoptosis, which occurs only when a specific set of genes is activated (Krantic et al., 2007; Sperandio et al., 2000). The processes of pyroptosis and pyronecrosis are similar forms of atypical programmed cell death, connected with the reaction of the immune system to infections. Their characteristic features include the involvement of Nod like receptors (NLR) in their induction, and formation of protein complexes termed inflammasomes (Bergsbaken et al., 2009; Ting et al., 2008). Finally, there are two cell death modalities such as excitotoxicity and Wallerian degeneration, which are characteristic exclusively for the cells of the nervous system (Kroemer et al., 2009). While the basic cell death types (apoptosis, necrosis, autophagy) in nervous system are relatively well known (for review: Lossi et al., 2005; Yuan et al., 2003; Yue et al., 2009), the knowledge about the mechanisms of this two atypical modes of death or partially degeneration in cells of the nervous system is still scarce, although there is a lot of extensive studies in this subject. It is also important, that recently published new recommendations of The Nomenclature Committee on Cell Death (Galluzzi et al., 2012) significantly alter the classification and nomenclature of the cell death processes, and excitotoxicity and Wallerian degeneration have not been taken into account there. Hence the purpose of this review is to describe the phenomena occurring in the nervous system: excitotoxicity and Wallerian degeneration as a process of cell death or partial degeneration.

Excitotoxicity

In 1969 Olney introduced the term "excitotoxicity" in his study on the effect of monosodium glutamate on brain functions in mice, to describe the neuronal death caused by excessive, or prolonged activation of receptors for the excitatory amino acids, among which glutamate plays the key role. Similar observations were made in regard to the ionotropic, and to a lesser extent metabotropic receptors (Casson and Franzo, 2006; Deng et al., 2006; Montoliu et al., 2002). It should be underlined, that the glutamate receptors are divided into two groups: ionotropic glutamate receptors (iGluR), connected with ion channels, whose activation leads to permeability of these channels to bivalent cations (such as Ca^{2+}), and metabotropic glutamate receptors (mGluR), whose activation leads to mobilization of the endogenous calcium pool via mechanisms dependent on the GTP-binding protein (Duncan, 2009). The iGluR are ligand-gated ion channels mediating the fast synaptic transmission (Casson and Franzo, 2006). They are characterized by their selective affinity to specific agonists (ligands), such as: *N*-methyl-D-aspartate to the NMDA receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid to the AMPA receptor (AMPAR), and kainic acid to the KA receptor (KAR) (Hynd et al., 2004; Vincent and Mulle, 2009). In case of the metabotropic glutamate receptors, they are divided into three groups (mGluRI-mGluRIII) on the basis of their amino acids sequence, sensitivity to their agonists, and the ability of signal transduction (Hynd et al., 2004). The mGluRs were not shown to play an important role in the excitotoxicity, although their involvement in this process was described in the white matter, and to smaller extent in the grey matter of CNS (Casson and Franzo, 2006). Studies of Deng and coworkers (2006) indicate, that this process can concern not only neurons, but also oligodendrocytes. The NMDA receptor is best known for its function, as well as its role in excitotoxicity. NMDAR shows

higher permeability to calcium cations than the two other ionotropic receptors, which is the reason for its greater ability to induce the Ca^{2+} cytosolic overload, leading to cell death (Duncan, 2009; Hynd et al., 2004). Activation of these receptors causes deregulation of the ionic homeostasis in cells, mainly regarding a significant increase of Ca^{2+} ions, which leads to cell death (Casson and Franzo, 2006; Kroemer et al., 2009). It has been demonstrated that in addition to calcium ions, free radicals and nitrogen oxide are also involved in this process (Fig. 1). The intracellular events during the excitotoxicity are

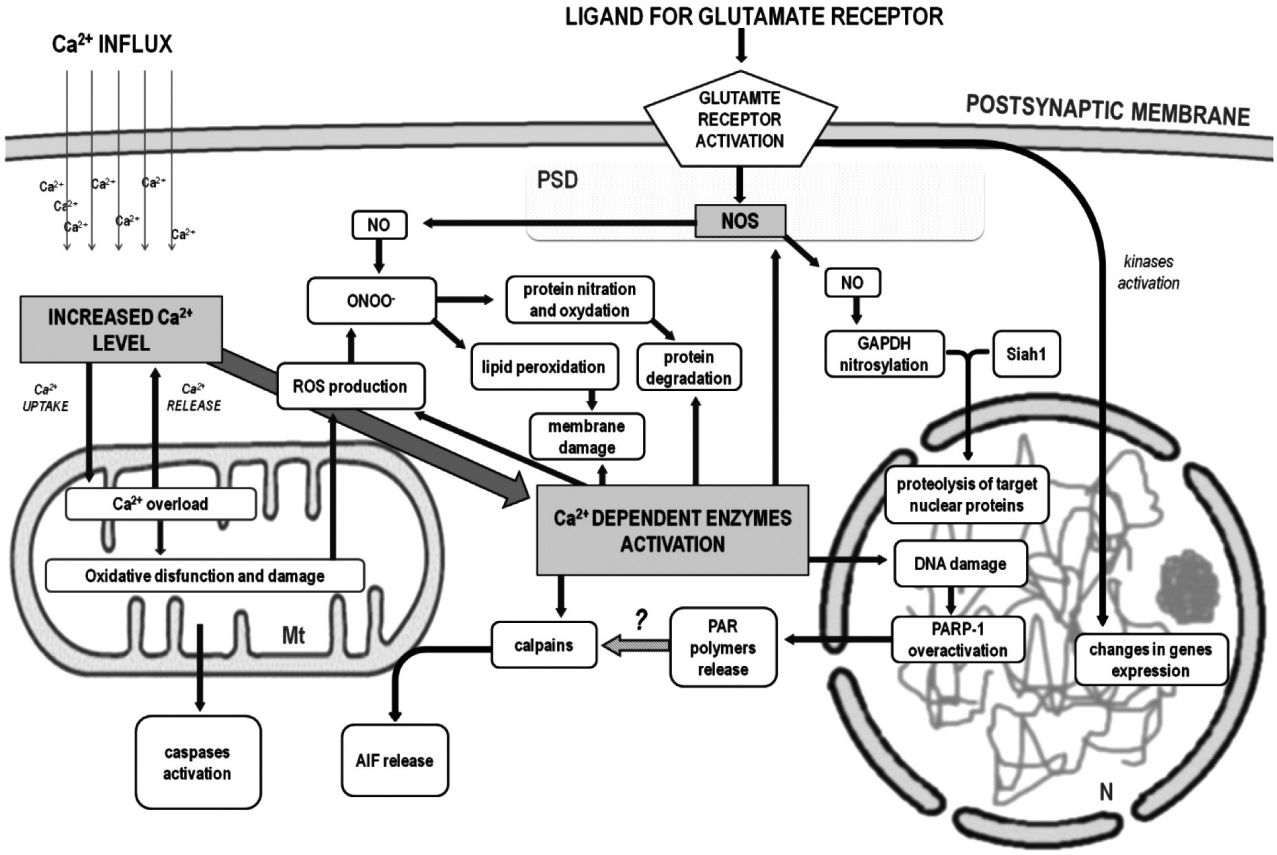

Fig. 1. - Overview on cell changes during excitotoxicity (Lau and Tymianski 2010; Metha et al., 2013; Flores-Soto et al., 2012).
Excessive activation of ionotropic glutamate receptors localized on postsynaptic membrane causes massive influx of calcium ions (Ca^{2+}) into the cell. Such increase of cytoplasmic calcium concentration can trigger a range of downstream events which lead to cell death. Excess of calcium ions is sequestered into mitochondria (Mt) and the overload of this organelles with calcium causes their dysfunction and damage. Dysfunctional or damaged mitochondria release calcium ions to cytoplasm as well as large amount of reactive oxygene species (ROS). Additionally metabolic dysfunction of mitochondrium can lead to caspase activation. Increased level of cellular calcium causes disrupted activation of calcium-dependent enzymes such as proteases including calpains, phospholipases, endonucleases, oxidases and nitric oxide synthase (NOS) which can cause production of ROS and/or damage of various cellular elements. On the other hand glutamate receptors are also linked to NOS through one of the post synaptic density protein (PSD), thus NOS can produce toxic levels of nitric oxide (NO) after glutamate receptor activation. NO can be toxic for neuron in at least two pathways. Firstly NO interacts with free radical superoxide to form peroxynitrite (ONOO-) which causes primarily protein nitration and oxidation and lipid peroxidation leading to cell death. Secondly NO can cause nitrosylation of glyceraldehydes 3-phosphate dehydrogenase (GAPDH), which subsequently binds to ubiquitin ligase (Siah1) and translocates to the nucleus (N) where proteasomes degrades Siah1 targets protein. Finally peroxynitrite can directly damage DNA. Damaged DNA activates poly-ADP-ribose polymerase 1 (PARP-1) leading to release polymers of poly-ADP-ribose (PAR) to cytoplasm, which in turns cause calpains activation by unknown mechanism (?) and subsequent release of apoptosis inducing factor (AIF) from mitochondria. Moreover activation of glutamate receptors can lead to protein kinases activation and subsequent downstream signaling causes changes in gene expression. The consequence of all these events can be disruption of cellular homeostasis leading to apoptotic or necrotizing cell death depending on intensity of stimuli.

still not fully understood, but it is now clear that in this process cysteine proteases, mitochondrial endonucleases, poly (ADP-ribose) polymerase 1 (PARP-1) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) could be required (Lau and Tymianski, 2010). The excitotoxicity is observed during brain damage, strokes, cardiac arrest, epilepsy, hypoxia, hypoglycemia, cerebral ischemia, and neurodegenerative diseases, such as: amyotrophic lateral sclerosis, and Huntington's, Alzheimer's, and Parkinson's diseases (Bevers et al., 2009; Montoliu et al., 2002; Wu and Li, 2009). For this reason many studies are conducted on the influx of different substances in excitotoxic neuronal death (Table I).

The latest reports indicate that the postsynaptic density proteins (PSD) also play an important function in excitotoxicity. PSD is a multiprotein structure connected with the postsynaptic membrane (Forder and Tymianski, 2009). Among several hundreds of proteins assembled within this large complex the ionotropic receptors are also found. These receptors form a complicated spatial network in conjunction with protein kinases, phosphatases, and cytoskeletal proteins, which localizes these proteins near the postsynaptic membrane, forming specific signaling pathways (Forder and Tymianski, 2009). Depending on the intensity of the initiating stimulus, the excitotoxicity may overlap with other types of cell death such as apoptosis and necrosis, however during this process the specific regulatory factors are not observed (Ientile et al., 2001; Kroemer et al., 2009). Because of these properties of excitotoxicity, NCCD does not classify this process as a separate, functional type of cells death, but define it as atypical cell death modality.

Wallerian degeneration

The process described as Wallerian degeneration was first described over 100 year ago by August Waller, who observed this specific phenomenon during his studies on lesioned hypoglossal, and glossopharyngeal nerves in frogs (Koeppen, 2004). The Wallerin degeneration (WD) takes place after axonal rupture in both central, and peripheral nervous systems (CNS and PNS), although in case of the CNS the process progresses in a much slower rate (Koeppen, 2004; Raff et al., 2002). The part of the axon, which was detached from the neuron's cell body in the course of the injury, is degenerating in a characteristic manner, through degradation of the endoplasmic reticulum and neurofilaments by ion-sensitive proteases, such as: calpains. Swelling of axonal mitochondria occurs, leading to their lysis, and the whole damaged part of the axon, separated from the neuron, is disintegrated into smaller pieces, which are removed by macrophages and Schwann cells in the PNS, or by activated microglia in the CNS (Luo and O'Leary, 2005; Raff et al., 2002; Wang et al., 2009; Yamagishi et al., 2005). It is thought, that the astrocytes may also be involved in phagocytosis of the damaged axons, and subsequently proliferate in order to fill in the space left after the phagocytized neural fibers (Wang et al., 2009). Even though the Wallerian degeneration has been known for a long time, the molecular mechanism of this process remains unrevealed, however it has been noted that it is caspase-independent, and it proceeds independently of the neuron death (Koike et al., 2008; Saxena and Caroni, 2007; Yamagishi et al., 2005). Additionally, after a discovery of spontaneous mouse Wld^s mutants (Wallerian degeneration slow) in 1989, showing significantly delayed and protracted WD, the perception of the process changed, and nowadays it is no longer considered as passive, caused by deprivation of nutrients supplied by the neuron's cell body, but as an active program of axonal degeneration (Hilliard, 2009). This breakthrough discovery provided many new data and expanded the knowledge on the Wallerian degeneration (Koeppen, 2004). It has been shown, that the mutation in Wld^s mice results from a tandem triplication of a 85 kb fragment within chromosome 4, causing formation of a new chimeric protein Wld^s composed of a complete amino acid sequence of nicotinamide mononucleotide adenylyl transferase (Nmnat), N-terminal 70 amino acids fragment of Ufd2 protein (ubiquitin fusion degradation protein 2), and a short peptide sequence that joins the two proteins together (Hilliard, 2009; Koike et al., 2008). The effect of this protein on the WD has been a subject of many extensive studies in the recent years. It has been reported that the Wld^s protein is expressed only in the nuclei, and is not found in axons, which suggests that this protein may regulate the expression of other proteins that initiate the Wallerian degeneration (Saxena and Caroni, 2007). The role of

Table I. - Effect of selected factors on the process of excitotoxicity.

Examined factor	Material	Glutamate receptor type	Results summary	Reference
ConantokinG (CGX-1007)-antagonist of NR2B subunit of NMDAR	Hippocampal brain slice cultures from Sprague Dawley rat pups	NMDAR	This factor inhibited NMDA-induced neuronal excitotoxicity through the blocking of different subtypes of NMDAR.	Alex et al., 2011
KN93, tat-AIP, tat-CN21-inhibitors of calcium-calmodulin (CaM)-dependent protein kinase II (CaMKII)	Embryonic cortical neuron culture from Sprague Dawley rat pups	NMDAR	Inhibition of CaMKII activity in cells exposed to NMDA, shortly after stimulation of the substance protects neurons against excitotoxicity, increasing their survival. While the permanent loss of the kinase activity increase susceptibility of neurons to glutamate caused excitotoxicity.	Ashpole and Hudmon, 2011
Tumor necrosis factor α (TNF-α)	Cerebella from postnatal 9-12 day old Sprague Dawley rats	AMPAR	This factor has intensified the excitotoxicity in the examined cells exposed to AMPA. In these cells, there was increased activity of calpains caused by increased influx of calcium ions.	Bliss et al., 2011
Vascular Endothelial Growth Factor (VEGF-A)	Motor neurons culture from Wistar rats	AMPAR	This factor prevented excitotoxicity involving AMPA receptors, and its mechanism of action was to intensifying transcription of GluR2 subunit of AMPA receptor, which meant that the receptor was not permeable to calcium ions.	Bogaert et al., 2010
Lithium	Organotypic slice culture of spinal cord from E16 chick embryos	KAR	This factor prevented the excitotoxicity with participation of KA receptor, but also caused large cytopathic changes and did not prevent apoptosis. He acted by blocking GSK-3β.	Caldero et al., 2010
Cationic liposomes associated to transferrin (Tf-lipoplexes) with anti-c-Jun siRNAs	Primary mouse embryonic cortical neurons from: C57/BL6 mice for *in vitro* studies; C57BL/6 mice for *in vivo* studies	KAR	This factor effectively inhibited the expression of c-Jun after KA stimulation, which resulted in reduced neuron death both in vitro and in vivo. These results suggest that the factor can be an effective therapeutic tool for treatment of neurodegenerative diseases which are accompanied by excitotoxicity.	Cardoso et al., 2010
2-methyl-6-(phenylethynyl)-pyridine (MPEP)-mGlu5 receptor antagonist	Mixed spinal cord cultures (MSCs) from 13-15 old Wistar rat embryos; astroglial cell cultures from spinal cord of Wistar new born rats	AMPAR	Prolonged pre-exposure to the selective mGlu5 receptor antagonist, MPEP, protected motor neurons (MNs) in MSCs against AMPA toxicity. This probably has happened due to reduction of brain derived neurotrophic factor (BDNF) expression and down-regulation of GluR1 subunits of AMPA receptors. Blocking of mGlu5 could be a novel target for treatment MNs disorders, for example ALS.	D'Antoni et al., 2011
L-theanine-the major amino acid from green tea	Human neuroblastoma transgenic cell line SH-SY5Y with expression of APP Swedish mutation as an *in vitro* model of Alzheimer's disease	NMDAR	This substance has protected cells using for experiment against L-glutamate-induced excitotoxicity. It was shown that L-theanine acts as a natural antagonist of glutamate and prevents the increase of amyloid β secretion induced by the overactivation of NMDAR. Thus it could be using as a prophylaxis and treatment agent in Alzheimer's disease.	Di et al., 2010

continued

Table I. - Effect of selected factors on the process of excitotoxicity.

Examined factor	Material	Glutamate receptor type	Results summary	Reference
Rosuvastatin (RST)	Primary cortical neurons from E18 Sprague-Dawley rat's embryos	NMDAR	Three-day RST pretreatment has protected cultured neurons against L-glutamate-induced excitotoxicity. Mechanisms of protection has likely involved reduced Ca^{2+} influx and decreased superoxide production.	Domoki et al., 2010
Idazoxan, 2-(2-benzofuranyl)-2-Imidazoline (2-BFI) imidazoline I_2 receptor antagonists	Primary cortical neurons cell culture from E15-E16 CD1 mice embryos	NMDAR	Idazoxan and 2-BFI reversibly block intracellular calcium influx in cortical neurons and are neuroprotective against glutamate toxicity. This substances can be examined as a therapeutics to treat excitotoxicity-mediated neurological disorders and they are expected to have minimal side effects toward NMDA receptor normal physiological functions.	Jiang t al. 2010
Lenti virus expressing Meteorin-newly identified neurotrophic factor	Left striatum of 2-3 months old Sprague-Dawley rats male; *in vivo* studies	NMDAR	Meteorin has protected striatal neurons against quinolinic acid mediated excitotoxicity and it could be using after additional examination as a novel therapeutic molecule for disorders with associated excitotoxicity such as Huntington disease.	Jorgensen et al., 2011
Tacrolimus (FK506)-immunosuppressant	Organotypic hippocampal slice cultures from 6-7 days old Sprague-Dawley rats	Not shown	Low dose of FK506 caused protective effect against KA-induced cell death in organothypic hippocampal slice cultures. FK506 reduced ROS level by SOD activation and apoptosis by phospho-Akt activation.	Lee at al. 2010
Ghrelin: an endogenous ligand of growth hormone secretagogue receptor 1a	Organotypic rat spinal cord cultures from 8-day-old Sprague-Dawley rat pups	Not shown	Treating with ghrelin inhibited spinal cord motoneuron death induced by chronic glutamate excitotoxicity. This substance activated ERK1/2 and Akt in motoneurons, and its protective effect was mediated by the MAPK and PI3K/Akt pathways. It was also shown, that ghrelin-induced Akt signaling is associated with downstream inhibition of GSK-3β in motoneurons.	Lim et al., 2011

Akt: protein kinase B; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; CNS: central nervous systems; ERK1/2: extracellular-signal-regulated kinase ½; GSK-3β: Glycogen synthase kinase 3β; iGluR: ionotropic glutamate receptor; KA: kainic acid; KAR: kainic acid receptor; MAPK: mitogen activated protein kinase; mGluR: metabotropic glutamate receptors; NCCD: Nomenclature Committee on Cell Death; NMDA: *N*-methyl-D-aspartate; NMDAR: *N*-methyl-D-aspartate receptor; Nmnat: nicotinamide mononucleotide adenylyl transferase; PCD: programmed cell death; PI3K: Phosphatidylinositol 3-kinase; PNS: peripheral nervous systems; PSD: postsynaptic density proteins; Ufd2: ubiquitin fusion degradation protein 2; UPS: ubiquitin proteasome systemv; WD: Wallerian degeneration; Wld^S: Wallerian degeneration slow

each fragment of the chimeric Wld^S protein has been examined in regard to WD induction, and it has been established that an overexpression of the nicotinamide mononucleotide adenylyl transferase (Nmnat) significanty delays this process, however the effect is less pronounced in comparison to the effect of the full Wld^S protein. Many hypotheses have been proposed regarding the delay of WD by Nmnat, suggesting that this enzyme may have more than one function. Nmnat is a key regulatory enzyme in the biosynthesis of the nicotinamide adenine diculeotide (NAD+), which is essential for biosynthesis and regulation of ATP. Nmnat is also involved in regulation of gene expression by interactions with histone deacetylase (HDAC), and it regulates microtubules stability via interactions with sirtuin

(SIRT-2) (Koike et al., 2008). So far, it has not been determined whether the protective effect of Nmnat overexpression results primarily from the improvement of cell metabolism, or is caused by other factors, such as Nmnat's interactions with other proteins (Koike et al., 2008). Analysis of the second part of the mutated Wld^s protein – the N-terminal 70 amino acids fragment of Ufd2 did not show its direct protective function in axons (Koike et al., 2008). However, it has been reported that this part of Ufd2 protein binds directly to VCP/p97 (vasolin-containing protein), which plays a key role in the ubiquitin proteasome system (UPS) (Hilliard, 2009). In fact, other studies revealed that the Wallerian degeneration depends on the activity of UPS protein complex, since inhibition of UPS counteracts the process (Saxena and Caroni, 2007). In order to block WD, the inhibition of UPS activity must, however, occur 2-3 hours before axonal lesion, suggesting that this protein complex regulates only the early stages of axon degeneration (Luo and O'Leary, 2005). Other studies additionally point at the involvement of other proteins, such as: interleukin-6 (IL-6) (Lee et al., 2009), Rho GTPases (Yamagashi et al., 2005), TNF (tumor necrosis factor) (Uceyler and Sommer, 2006) in different stages of WD. Wallerian degeneration is not a typical cell death mechanism, since neurons undergoing this process remain alive, and therefore the WD is considered atypical (Kroemer et al., 2009).

Concluding remarks

Cell death is a process occurring very commonly in living organisms, during their developmental processes, normal physiology, as well as pathological conditions. The cells may undergo cell death via many pathways and mechanisms, among which four typical pathways are relatively best described, although more research is needed in this matter. The discovery of new, atypical forms of cell death has brought a lot of interesting information to this field of research. Nervous system plays an extremely important function in living organism, determining its contact with environment, but at the same time its regenerative abilities are very limited. It is therefore important to study and describe processes involved in death of the nervous system cells, and discover their causes. In the future it is possible that this type of studies will bring progress in the treatment of many dangerous diseases of the nervous system, including neoplasms and neurodegenerative diseases.

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