Progress in Neurobiology xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Progress in Neurobiology

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

Role of the ubiquitin–proteasome system in brain ischemia: Friend or foe?

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ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 8 October 2013
Accepted 15 October 2013
Available online xxx

Keywords:
Ubiquitin–proteasome system
Brain ischemia
Excitotoxicity
Proteasome inhibitors

ABSTRACT

The ubiquitin–proteasome system (UPS) is a catalytic machinery that targets numerous cellular proteins for degradation, thus being an essential system to control a wide range of basic cellular processes and cell survival. Degradation of intracellular proteins via the UPS is a tightly regulated process initiated by tagging a target protein with a specific ubiquitin chain. Neurons are particularly vulnerable to any change in protein composition, and therefore the UPS is a key regulator of neuronal biology. Alterations in UPS activity may induce pathological effects, ultimately leading to neuronal cell death. Brain ischemia triggers a complex series of biochemical and molecular mechanisms, such as an inflammatory response, an exacerbated production of misfolded and oxidized proteins, due to oxidative stress, and the breakdown of cellular integrity mainly mediated by excitotoxic glutamatergic signaling. Brain ischemia also damages protein degradation pathways which, together with the overproduction of damaged proteins and consequent upregulation of ubiquitin-conjugated proteins, contribute to the accumulation of ubiquitin-containing proteinaceous deposits. Despite recent advances, the factors leading to deposition of such aggregates after cerebral ischemic injury remain poorly understood. This review discusses the current knowledge on the role of the UPS in brain function and the molecular mechanisms contributing to UPS dysfunction in brain ischemia with consequent accumulation of ubiquitin-containing proteins. Chemical inhibitors of the proteasome and small molecule inhibitors of deubiquitinating enzymes, which promote the degradation of proteins by the proteasome, were both shown to provide neuroprotection in brain ischemia, and this apparent contradiction is also discussed in this review.

Abbreviations: 3-MA, 3-methyladenine; 15d-PHJ$_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin J2; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid; AMPAR, AMPA receptors; APC/C, anaphase-promoting complex/cyclosome; APV, D-aminophosphonovalerate; ax^J, ataxic mouse; BBB, blood–brain barrier; BCO, bilateral carotid occlusion; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; Cdk5, cyclin-dependent kinase-5; CHOP, CHOP (C/EBP homologues protein); CP, catalytic particle; DUB, deubiquitinating enzymes; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GRP78, glucose-regulated protein 78; HECT, E6-AP Carboxyl Terminus; HERP, homocysteine-inducible, ER-stress inducible protein; KA, kainate; MCAO, middle cerebral artery occlusion; Mdm2, murine-double minute 2; mESPC, mini excitatory post-synaptic current; NCX3, sodium–calcium exchanger type-3; Mib2, Mind Bomb-2; Nedd4, neuronal-precursor cell-expressed developmentally downregulated gene 4; NMDA, N-methyl-D-aspartate; NMDAR, NMDA receptors; NOS, nitric oxide synthase; eNOS, endothelial NOS; OGD, oxygen and glucose deprivation; PDI, protein disulphide isomerase; PERK, PKR-like ER kinase; Pru, pleckstrin-like receptor for ubiquitin; PSD, post-synaptic density; PSD-95, post-synaptic density associated protein 95; RING, Really Interesting New Gene; RP, regulatory particle; tPA, tissue plasminogen activator; TRPM, transient receptor potential melastatin; UBA, ubiquitin associated domain; UCH, ubiquitin C-terminal hydrolases; UIM, Ubiquitin-Interacting Motif; UPR, unfolded protein response; UPS, ubiquitin–proteasome system; USP, ubiquitin specific protease; VGLUT, vesicular glutamate transporters.

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0301-0082/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.pneurobio.2013.10.003

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1. Brain ischemia

Brain ischemia is a leading cause of death and disability worldwide, resulting from a reduction in the blood flow to the brain. This leads to a deprivation of oxygen and glucose, and cell death is the fatal end caused by the reduction of the fuels available for the metabolism of the cells. Brain ischemia may be caused by cardiac arrest (global ischemia, affecting the entire brain) or by ischemic stroke (focal ischemia, which targets a specific brain region).

Ischemic stroke occurs as a result of the occlusion of a blood vessel supplying blood to the brain by a thrombus or an embolus (Doyle et al., 2008; Roger et al., 2012). In this condition, the region of the brain that is most affected, the ischemic core, fully depends on oxygen and glucose provided by the affected blood vessel, while the penumbra region, the area surrounding the infarcted core, is not as affected due to a limited supply of components required for the metabolism derived from the collateral circulation. Glucose is the main substrate for cerebral energy production (Hofmeijer and van Putten, 2012) and during stroke the oxygen carried by the blood is much less than that required for complete oxidation of its content of glucose. Under these conditions glycolysis may persist after oxygen has been depleted, but the reduction of oxidative metabolism of glucose leads to decreased ATP levels, while ADP and AMP levels increase (Hertz, 2008), causing a disruption of ionic homeostasis (Hansen, 1985), opening of anion channels (Kimelberg and Mongin, 1998), plasma membrane depolarization (Lipton, 1999), release of glutamate through astrocytic hemichannels (Ye et al., 2003) and downregulation of glutamate transporters (Harvey et al., 2011). The impairment of glutamate transporters, in addition to their operation in the reverse mode, leads to an accumulation of glutamate in the extracellular space (Grewer et al., 2008) and a consequent overactivation of postsynaptic glutamate receptors. Under these conditions, necrotic cell death occurs at the core region, while in the penumbra region the availability of ATP allows a delayed cell death by apoptosis (Broughton et al., 2009).

The hippocampus is particularly vulnerable to brain ischemia, but distinct responses are typically observed in the different hippocampal subregions. The CA1 region is highly sensitive to transient ischemia followed by reperfusion, but this neuronal population die far later after ischemic insult, a process referred to as delayed neuronal death (Kirino, 1982; Pulsinelli et al., 1982a). This is a unique type of cell death that progresses despite complete recovery of metabolic parameters, such as regional blood flow, glucose metabolism and tissue ATP content (Kirino, 1982; Pulsinelli et al., 1982b; Mies et al., 1990), but the molecular mechanisms involved are yet to be clearly understood.

1.1. Role of glutamate

During stroke, membrane depolarization due to ATP breakdown leads to an increase in the release of glutamate, and the lack of energy blocks the reuptake of the excitatory amino acids at the synapse, leading to an extracellular accumulation of glutamate (Rossi et al., 2000; Grewer et al., 2008). The reversal of the glutamate transporters under these conditions (Rossi et al., 2000; Grewer et al., 2008) further contributes to the extracellular accumulation of glutamate, with a consequent toxic overactivation of postsynaptic glutamate receptors (excitotoxicity) (Olney, 1969; Simon et al., 1984; Choi et al., 1987; Ferreira et al., 1996, 1998; Martel et al., 2012). Upon oxygen and glucose deprivation (OGD), a well established in vitro model of global ischemia, glutamate is massively released by neurons, and the resulting increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Goldberg and Choi, 1993) causes a delayed neuronal cell death (*calcium overload hypothesis*) (Manev et al., 1989). Ca^{2+} uptake and neuronal cell death can be prevented by the NMDA (N-methyl-D-aspartate) receptor (NMDAR) antagonist D-aminophosphonovalerate (APV) (Goldberg and Choi, 1993). In addition, the volume of microinfarcts induced by occlusions of individual penetrating arterioles or venules in the rat brain cortex could be reduced by administrating meantime 30–45 min after the occlusion, ameliorating perceptual deficits (Shih et al., 2013). These data indicate that NMDAR, characterized by their high Ca^{2+} permeability, are the link between glutamate, Ca^{2+} and neuronal cell death. On the other hand, it was shown that blocking NMDAR with MK-801 resulted in a decreased density of healthy cells in the dentate gyrus (Gould et al., 1994), indicating that a moderate flow of Ca^{2+} ions through NMDARs is beneficial for neurons, while Ca^{2+} overload, linked with an excessive NMDAR activation is deleterious (NMDAR paradox) (Hardingham and Bading, 2003).

Not all NMDAR contribute to neuronal cell death in excitotoxicity, since the synaptic and extrasynaptic receptor populations

Please cite this article in press as: Caldeira, M.V., et al., Role of the ubiquitin–proteasome system in brain ischemia: Friend or foe? Prog. Neurobiol. (2013), http://dx.doi.org/10.1016/j.pneurobio.2013.10.003