

● PERSPECTIVE

Estrogen/Huntingtin: a novel pathway involved in neuroprotection

Neurodegenerative diseases (NDs) include more than 600 disease entities that are characterized by loss of specific neurons located in anatomically related functional areas which progressively lead to motor and cognitive deficits. The pathogenesis of NDs involves mitochondrial dysfunction/oxidative stress, programmed cell death or abnormal protein aggregation, trafficking, and/or degradation. In most cases, the end stage neuropathology is characterized by a highly specific distribution of abnormal protein aggregates in disease specific patterns in the affected neuronal populations. These proteins include β -amyloid and TAU in Alzheimer's disease (AD), α -synuclein in Parkinson's disease (PD), and huntingtin (Htt) polyglutamine (poly-Q) elongation in Huntington's disease (HD) (Maiese, 2015). NDs result in disability and death for more than 30 million individuals worldwide and the number of individuals afflicted is expected to increase with the increased life span of the global population. Although clinical treatments for NDs have progressed over the years with some promising results, the availability of treatments that can limit or prevent NDs remains limited (Maiese, 2015).

A challenging approach to the treatment of NDs could derive from the study of the role and the action mechanisms of endogenous substances which neuroprotective effects are well known. Estrogens, gonadal steroids, seem to be good candidates for these studies. Indeed, a growing number of evidence concerning structural, cellular, and molecular differences in diverse male and female brain regions could explain male and female diverse response to environmental challenges and different vulnerabilities to behavioral and neurological disorders. Striking differences in symptomatology, prevalence, progression, and severity between sexes occur in several neurodegenerative diseases (Miller et al., 2005). Epidemiological data clearly show that the incidence and prevalence of PD is 1.5 times higher in men than in women, whereas AD becomes prevalent in women only after 90 years of age (23.6% women *versus* 17.6% men) (www.epicentro.iss.it). Although potential sex differences concerning HD are poorly defined, few reports suggest that the age of onset of HD is higher and the course of disease is more moderate in women compared with men. In addition, animal models of HD indicate sex-related differences in the HD phenotype (Bode et al., 2008; www.epicentro.iss.it). As a whole, these evidences point to a substantial beneficial influence of 17 β -estradiol (the most active within estrogens; E2) against the development and progression of neurodegenerative diseases.

Besides the female reproductive tract, E2 exerts multiple and diverse actions in male and female brain throughout the life span, beginning during development and continuing on into senescence. In fact, E2 is a potent trophic factor that influences development, differentiation plasticity, cell survival, cell migration, neuronal somatic and dendritic growth, and synapse formation. Notably, E2 displays neuroprotection against β -amyloid toxicity and oxidative stress-induced neuronal death (Fiocchetti et al., 2012). Moreover, E2 protects neurons against apoptosis by reducing the production of reactive oxygen and nitrogen species (ROS and RNS, respectively) and/or facilitating ROS and

RNS scavenging, as well as inhibiting the neurotoxic effects of oxidized low density lipoproteins and glutamate, maintaining the Ca²⁺ homeostasis, regulating pro-apoptotic caspase activities, and maintaining mitochondrial membrane integrity (Gillies and McArthur, 2010; Fiocchetti et al., 2012). E2 also influences higher order brain functions such as memory formation, mood, neurodevelopment, pain sensitivity, cognition, motor coordination, and neurodegeneration by promoting neuronal survival and tissue integrity (Srivastava et al., 2013).

E2 effects are mediated by the two estrogen receptors subtypes (ER α and ER β), members of nuclear receptor family, which are widely distributed in several male and female brain regions including hypothalamus, hippocampus, striatum and cortex (Mitra et al., 2003). To complicate this picture, in 1997, a G protein-coupled receptor, termed GPR30, was cloned and established as another E2-binding membrane receptor. Similar to ERs, GPR30 is expressed throughout the brain where it could also play a physiological role in regulation of brain functions. With the identification of these three different receptors, the understanding of the brain effects of E2 has become increasingly complex. To render more complex this picture, ER-mediated cellular responses to E2 have been loosely grouped into two interconnected categories: nuclear and rapid extra-nuclear signals. It is now known that both ER subtypes share the same molecular action mechanisms which depend on their intracellular localization.

In our laboratory we recently demonstrated that E2, *via* the synergy between extra-nuclear and nuclear action mechanisms, induces a 4-fold increase of levels of a new globin neuroglobin (Ngb) in human neuroblastoma cell line, mouse primary hippocampal neurons, as well as in mouse primary astrocytes (Fiocchetti et al., 2013). Remarkably, Ngb silencing experiments demonstrated that this protein is necessary for E2-induced protective effects against H₂O₂-toxicity. Our experiments indicate that upon E2 stimulation Ngb localizes mainly into mitochondria where it binds to mitochondrial cytochrome c. An oxidative insult with H₂O₂ increases the E2-mediated Ngb association with the mitochondrial cytochrome c, thus reducing the cytochrome c release into the cytosol. As a consequence, a decrease of caspase-3 activation and, in turn, of the apoptotic cascade activation occurs (De Marinis et al., 2013). These data strongly sustain the pivotal role played by over-expression and mitochondrial localization of Ngb in E2-induced protective effects against oxidative stress-induced neurotoxicity.

However, the existence of a possible carrier protein responsible of Ngb translocation from nucleus to mitochondria is completely unknown. A possible good candidate as Ngb carrier is Htt. Htt is a 3,144 amino acid protein ubiquitously expressed. Htt is a soluble protein placed close to the intracellular compartments including Golgi complex, mitochondria, endoplasmic reticulum and nucleus and connected with vesicles, endosomal compartments and microtubules next to neurites and synapses (Zuccato et al., 2010). Near the Htt N-terminus there is a poly-Q region starting from the eighteenth amino acid residue and creating a polar zipper followed by a poly-P region that could justify Htt solubility; going along there are HEAT repeats. These motifs are thought to form a flexible scaffold that supports dynamic protein-protein interactions (Zuccato et al., 2010). Finally at the C-terminal of the protein a nuclear export signal (NES) and a nuclear localization signal (NLS) are present. The broad expression and the complex amino acid sequence of Htt render difficult the search for Htt function, but several studies revealed



a critical role for this protein in several cellular activities including transcriptional regulation, vesicular shuttling, embryonic development, regulation of apoptosis and autophagy (Zuccato et al., 2010). As a whole, Htt displays several beneficial activities in the mature brain that lead us to investigate the hypothesis that Htt could take part of E2 neuroprotective processes. Surprisingly, E2 increases Htt levels in a time and dose dependent manner (Nuzzo et al., 2015). This effect, detectable by 6 hours until 24 hours of E2 treatment, is not activated by androgens and relies on both ER α genomic and rapid pathways to up-regulate Htt levels (Nuzzo et al., 2015). The rapid process responsible for this action is triggered by ER α -mediated Akt activation. These results have been further confirmed in hippocampal and striatal tissues of male and female Wistar rats. In particular, in the striatal nuclei, female rats exhibit higher Htt levels than male rats (Nuzzo et al., 2015). In addition, adult female rats express high Htt levels compared to the pre-puberal animals in line with the highest E2 circulating levels of females rats after puberty (Nuzzo et al., 2015). Notably, E2-induced Htt up-regulation is concomitant with the increased Ngb: Htt association in the mitochondria (Nuzzo and Marino, unpublished results) and with the neuron protection against H₂O₂-induced apoptosis. On the other hand, E2-induced Ngb up-regulation and protection against apoptosis was strongly prevented in Htt-silenced neuroblastoma cells (Nuzzo et al., 2015).

The expanded, unstable CAG repeat sequence in the gene codifying for Htt elongates the segment of glutamine residues in Htt Poly-Q region bearing an oversize glutamine tract responsible for HD. This mutation endows Htt with toxic properties lethal for nervous cells: the mutated part of the protein can be cleaved off the entire protein to form cell inclusions; in addition mutant Htt elicits transcriptional dysregulation, proteasomal, autophagic and metabolic deficits, mitochondrial dysfunctions, oxidative stress, apoptosis, neuroinflammation, and consequent neurodegeneration (Zuccato et al., 2010). These deleterious functions first damage the spiny-projection GABAergic neurons of the striatum and then extend their action on other brain areas including cerebellar cortex, thalamus and cerebellum. Although it is established that HD occurs as a consequence of this mutation, some studies also point to the loss of normal Htt physiological activities as a crucial aspect of the disease pathogenesis and selectivity (Zuccato et al., 2010). The preliminary results obtained by our group in mouse wild type striatal neurons (STHdh^{Q7}) and in striatal neurons containing mutated Htt (STHdh^{Q111}) show that E2 modulates Htt and Ngb levels as well as the interaction between Htt and Ngb in wild type cells, but the hormone effect are absent in STHdh^{Q111} neurons (Nuzzo and Marino, unpublished results). As a consequence, lower levels of Ngb are present in mitochondria of STHdh^{Q111} cells with respect to wild type cells and E2 does not protect STHdh^{Q111} cells against H₂O₂-induced apoptosis as demonstrated by the increasing of apoptotic nuclei number and by the increased activity of caspase-3/7. Thus, mutated Htt loses its ability to carry the anti-apoptotic Ngb into the mitochondria (Nuzzo and Marino, unpublished results).

As a whole, these results suggest the existence of a novel neuroprotective axis, consisting of E2 induction of Htt and Ngb expression levels by two different and parallel mechanisms with a convergent outcome: the arrest of apoptotic cascade and the neuron survival against oxidative stress injury. The first step depends on the mediation of ER α and culminates in Htt up-reg-

ulation. Htt expression results crucial for the realization of the other step, consisting in the increase of Ngb expression through ER β . Future experiments aimed to elucidate the structural, biochemical, and functional bases of E2-induced Htt and Ngb up-regulation and association will open new scenarios in the field of HD treatment inserting the up-regulation of Ngb levels as a possible new therapeutic target of HD.

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Maria Teresa Nuzzo, Maria Marino*

Department of Science - Division of Biomedical Sciences and Technologies, University Roma Tre, Viale Guglielmo Marconi, Rome, Italy

*Correspondence to: Maria Marino, Ph.D.,

maria.marino@uniroma3.it.

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orcid: 0000-0002-6314-3397 (Maria Marino)

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