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Interplay between Calcium dependent proteolysis and neuronal NO production is involved in neurodegeneration

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Brain damages, associated to aging or neurodegeneration has been attributed to the production of reactive species or to an overload of Ca^{2+}. However, both oxygen and Ca^{2+} play important physiological functions and it is difficult to define when the cell responses elicited by these compounds can acquire pathological aspects. We have studied the possible physiological interplay between oxygen and Ca2+ dependent systems, as well as the transition to a possible pathological role.

In resting human neuronal cells, nitric oxide synthase (nNOS) is present in its native 160 kD form in a quiescent state predominantly co-localized on the plasma membrane, via its PDZ domain, with N-methyl-D-aspartate receptor (NMDA-R) and in tight association with heat shock protein 90 (HSP90). Following exposure to Ca^{2+}-ionophore or to NMDA, nNOS undergoes proteolytic removal of the PDZ domain being converted into a fully active 130 kD form. The newly generated nNO synthase form dissociates from NMDA-R and extensively diffuses into the cytosol in direct correlation with NO production. Intracellular redistribution and activation of nNOS are completely prevented in cells preloaded with calpain inhibitor-1, indicating that these processes are triggered by a concomitant activation of calpain. The role of calpain has been confirmed by immunoprecipitation experiments revealing that μ-calpain is specifically recruited into the NMDA-R-nNOS-HSP90 complex following calcium loading. To establish if these processes are related to neurodegenerative disorders, we examined the intracellular functional state of the calpain/calpastatin system in -G93A(+) SOD1 transgenic mice, an animal model of amyotrophic lateral sclerosis, in order to establish if and how uncontrolled activation of calpain can be prevented *in vivo* during the course of prolonged $[Ca^{2+}]_i$ elevation. Calpain activation is more extensive in motor cortex, in lumbar, and sacral spinal cord segments compared to the lower or almost undetectable activation of the protease in other SNC areas. Direct measurements of the variations of Ca^{2+} levels shown that the degree of the protease activation is correlated to the extent of elevation of $[Ca^{2+}]_i$, always associated with diffusion of calpastatin from perinuclear aggregated forms into the cytosol and the formation of a calpain/calpastatin complex; 4) a conservative fragmentation of calpastatin is accompanied by its increased expression and inhibitory capacity in conditions of prolonged increase in $[Ca^{2+}]_i$. Thus, calpastatin diffusion and formation of the calpain/calpastatin complex, together with an increased synthesis of the inhibitor protein, represent a cellular defence response to conditions of prolonged dysregulation in intracellular Ca^{2+} homeostasis. In concomitance with the activation of calpain, in those brain tissues showing the alteration in Ca^{2+} homeostasis neuronal NO synthase is cleaved into its active form, lacking the PDZ domain. Thus, the prolonged disregulation of intracellular $[Ca^{2+}]_i$, accompanied by the production of large amounts of NO, could be the triggering event leading to the onset of a pathological state due to the combined action of these compounds. Altogether, these findings provide a new understanding of the *in vivo* molecular mechanisms governing calpain activation and NO production potentially useful for the development of new therapeutic approaches for neurodegenerative diseases.