CELLULAR AND SUBCELLULAR LOCALIZATION OF Na^+-Ca^{2+} EXCHANGER (NCX) 1, 2 AND 3 IN CENTRAL NERVOUS SYSTEM (CNS)

Magi Simona[1], Castaldo Pasqualina[1], Salucci Sara[3], Gobbi Pietro[3], Minelli Andrea[2] and Amoroso Salvatore[1]

[1]Section of Pharmacology, Department of Neuroscience, University "Politecnica delle Marche", Ancona and Institute of [2]Physiological and [3]Morphological Sciences, University of Urbino "Carlo Bo"

NCX plays a fundamental role in controlling homeostasis of intracellular Na^+ and Ca^{2+} ions. Three different NCX1-3 isoforms have been identified and all are expressed in CNS. NCX1 and NCX3 are involved in the development of permanent focal ischemia and in sensitizing neurons to NMDA-mediated excitotoxicity, while NCX2 in learning and memory processes.
In the present study, light and electron microscopic immunohistochemistry was used to investigate the cellular and subcellular localization of NCXs, in the cerebral cortex and hippocampus of adult rats. Results showed that NCXs were ubiquitously expressed in both brain areas: NCX1 immunoreactivity (ir) associated to small-sized puncta, while ir for NCX2 and NCX3 also in pyramidal and non-pyramidal neurons and in dendritic-like structures. Electron microscopic analyses showed that NCXs were expressed in neuronal dendrites and dendritic spines often contacted by asymmetric axon terminals, indicating that NCXs are well sited for buffering $[Ca]_i$ in excitatory postsynaptic sites. Besides neurons, ir for NCXs was observed in glial profiles notably in distal astrocytic processes in contiguity of synaptic structures. In addition, NCXs were expressed in perivascular astrocytic endfeet and in endothelial cells. Since NCX seems to be involved in the regulation of mitochondrial $[Ca^{2+}]$, we investigated its expression also in mitochondria. Quantitative analyses of NCXs ir revealed a conspicuous population of neuronal and astrocytic mitochondria labelled: 1) NCX2 resulted the most expressed isoform in both neocortex and hippocampus; 2) NCX3 labelled mitochondria were mostly found in neurons; 3) NCX1 labelled mitochondria were equally distributed in neurons and astrocytes in neocortex, while in the hippocampus they were found prevalently in neurons. Interestingly, most NCXs-expressing mitochondria were found in neuronal dendrites, often located beneath the plasmalemma and near postsynaptic sites. Collectively, ultrastructural analyses in cerebral cortex and hippocampus revealed that NCXs were expressed mainly in dendritic domains, particularly in distal dendrites and spines reached by asymmetric synapses, and in astrocytic processes which often ensheathe synapses. The widespread NCXs expression in heterogeneous cell types of neocortex and hippocampus *in situ* emphasizes the role played by the three exchanger isoforms in handling Ca^{2+} and Na^+ in both excitable and non-excitable cells. In addition, quantitative and qualitative immunocytochemical data indicate that all NCX isoforms might contribute to Ca^{2+} homeostasis in neurons and glial cells *in vivo*, also by handling mitochondrial Ca^{2+} in dendritic subplasmalemmal mitochondria.