CONTRIBUTION OF VARIOUS ISOFORMS OF Na^+/Ca^{2+} EXCHANGER IN Ca^{2+} HOMEOSTASIS IN PRIMARY BRAIN CULTURES

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Na^+/Ca^{2+} exchanger (NCX) is an ion transporter constituted by 11 transmembrane segments, distributed widely in heart, kidney, brain and other tissues. In the brain, NCX mRNA is abundant in the cortex, hippocampus, dentate gyrus, thalamus and cerebellum. Three mammalian isoforms of the NCX have been cloned and named NCX1, NCX2 and NCX3. Genes encoding NCX1 have been cloned from several tissues, whereas expression of other genes coding for NCX2 and NCX3 appears restricted to brain and skeletal muscle. Under normal conditions, NCX transports Na^+ into the cells and Ca^{2+} out (forward mode of operation) thus contributing to the maintenance of free Ca^{2+} ($[Ca^{2+}]_i$) homeostasis. However, under some conditions, such as when intracellular Na^+ ($[Na^+]_i$) is increased or the membrane is depolarised, NCX reverts its mode of operation moving Ca^{2+} into the cells and Na^+ out.

The aim of the present study was to evaluate the contribution of the different soforms of NCX in $[Ca^{2+}]_i$ homeostasis in primary brain cultures. In this aim, cortical and hippocampal neurons were prepared from brains of 15-17-days-old rat embryos and plated on coverslips precoated with poly-D-lisyne (20 g/ml). RT-PCR analysis was performed and the results showed that NCX1 and NCX2 isoforms were present in cortical neurones whereas all the three isoforms of NCX were detected in hippocampal and cerebellar neurones. NCX activity was monitored by measuring $[Ca^{2+}]_i$ on single cell using microfluorimetric technique with the fluorescent probe FURA 2-AM in experimental conditions know to activate NCX in the reverse mode of operation (extracellular Na^+ removal). In this conditions an increase of $[Ca^{2+}]_i$ was observed and this increase was due only to the activation of NCX since the glicosilate synthetic peptide NCX inhibitor (Glu-XIP; 10-100 M) completely abolished this $[Ca^{2+}]_i$ elevation.

To evaluate the contribution of the different NCX isoforms to the $[Ca^{2+}]_i$ homeostasis, neurones were incubated with antisense oligodeoxynucleotides (NCX AS-oligos) targeted to the 3'-untraslated region of NCX1, NCX2 and NCX3 mRNA at concentrations 0,5-2-5 M for 3, 6, 12, 24, 36 and 48 hours. NCX1 and NCX2 AS-oligos time- and dose-dependently inhibit NCX activation in cortical and in hippocampal neurones, whereas NCX3 AS-oligos did not. In parallel cultures, a set containing the same base composition, but with scrambled sequence (nonsense or NS-oligos) and a set of sense oligodeoxynucleotides (S-oligos) did not modify $[Ca^{2+}]_i$ increase induced by extracellular Na^+ removal.

In conclusion these results suggest that:

1. Cortical neurones express NCX1 and NCX2 isoforms;
2. Hippocampal and cerebellar neurones express all the three isoforms;
3. NCX1 and NCX2 participate to the control of $[Ca^{2+}]_i$ homeostasis in cortical and hippocampal neurones.