KNOCKING-DOWN AND KNOCKING-OUT OF NCKX2 GENE EXPRESSION EXACERBATE THE BRAIN INSULT INDUCED BY FOCAL ISCHEMIA

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Sodium/Calcium exchangers are neuronal plasma membrane transporters which, by coupling Ca2+ and Na+ fluxes across neuronal membranes, may play a relevant role in brain ischemia. The exchanger gene superfamily comprises two arms: the K+-independent (NCX) and K+-dependent (NCKX) exchangers. In the brain, 3 different NCX and NCKX family members have been described (1, 2, 3).
Previous studies showed that more than 60% of calcium extrusion was mediated by sodium calcium exchangers and that 90% of this exchange was NCKX-mediated (4). Recently, it has been shown that NCX blockade induces a worsening in neuronal damage, whereas its activation may provide neuroprotection in an in vivo model of focal ischemia (5). By contrast, the function of NCKX in cerebral ischemia has not been determined. Thus, the aim of the present study was to investigate the role played by NCKX2 in rodents subjected to permanent or transient middle cerebral artery occlusion, pMCAO and tMCAO.
To this purpose, NCKX2 mRNA and protein expression were evaluated in the ischemic core and in the remaining ipsilateral non-ischemic area at different times after pMCAO in rats, by means of real-time RT-PCR, in situ hybridization, Western Blot, and immunohistochemistry analysis. The results showed that, both in the ischemic core and in the periinfarctual area, NCKX2 mRNA and protein were downregulated. Interestingly, immunohistochemical experiments revealed that, although the NCKX2 immunoreactivity was globally reduced, some surviving cells, mainly localized in the pheripheral zone of the ischemic core strongly expressed NCKX2 protein.
The role of this protein in the development of ischemic damage was also assessed in ischemic rats in which NCKX2 expression was knocked-down by a specific antisense oligodeoxynucleotide (ODN) intracerebroventricularly injected or in ischemic knocked-out *nckx2*-/- mice. Both knocking-down and knocking-out NCKX2 expression by antisense strategy or genetic deletion drammatically increased the extent of the ischemic lesion in rats and mice subjected to pMCAO and tMCAO, respectively, showing that NCKX2 plays a pivotal role in the development of cerebral ischemia.
Overall, these results suggest that NCKX2 is involved in the progression of the ischemic lesion and may represent a potential target in the development of new therapeutic strategies for stroke treatment.