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Pattern expression of pro-apoptotic genes in neurons: Examination of the Clca1 gene under hypoxic/ ischemic conditions

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A recent gene chip analysis in our lab revealed the exceptional induction of the gene Clca1, which when over-expressed in hippocampal neurons, leads to an increased percentage of dead neurons. This study dealt with expression patterns of Clca1 after hypoxic/ ischemic conditions. QRT-PCR analysis showed, that both Clca1 and the control gene c-fos were induced in hippocampal neurons, exposed to OGD (oxygen glucose deprivation) test. Clca1 induction followed a strong kinetic result and was dependent on the establishment of a reperfusion time after OGD. Thus, the Clca1 expression pattern differed between continuous and intermittent hypoxia. These results indicate, that the ischemic length together with the duration of re-oxygenation in the recovery period, determines the magnitude of the genomic response. The in vitro data were confirmed by the animal stroke model of Middle Cerebral Artery Occlusion (MCAO) in mice. mRNA levels of both genes, Clca1 and c-fos, were significantly increased in the brains of animals applied to the stroke model in comparison to the sham-operated ones. Different brain tissue was more or less sensitive to ischemic conditions: While c-fos was strongly induced in hippocampal cells, only a moderate increase was measured in cortical neurons applied to OGD. These findings were affirmed in vivo. Although the MCAO model primarily affects the cortex, delayed enhancement of Clca1 induction was also detected in the hippocampus - underlining the sensitivity of Clca1 to ischemic/ hypoxic conditions. To determine the role of NMDA receptors in OGD-induced expression of Clca1 and c-fos, the non-competitive NMDA receptor antagonist, MK-801 was added to the OGD medium. MK-801 blocked the up-regulation of Clca1 and c-fos completely, indicating the important role of NMDA receptors in the control of both genes. NMDA receptors can be attributed to synapses but they are also present outside synaptic contacts. Since synaptic and extrasynaptic NMDA receptors differ fundamentally in the way they affect gene expression and neuronal fate, it was then investigated whether synaptic and extrasynaptic NMDA receptors differentially control OGD- induced gene responses. To isolate the effects of extrasynaptic NMDA receptors from those induced by synaptic NMDA receptors, a bicuculline/MK-801/TTX protocol was used. The induction of Clca1 by OGD was not compromised by selective blockage of synaptic NMDA receptors. In contrast, c-fos expression was dramatically reduced. This strongly suggests that the activation of extrasynaptic NMDA receptors triggers the induction of Clca1, while c-fos expression depends on synaptic NMDA receptors. The regulation of Clca1 and c-fos also differed in their sensitivity to TTX. c-fos induction was reduced in the presence of TTX during OGD. These findings may be explained by the effect of OGD on neuronal network activity: A strong increase of spike frequency, mediated by synaptic NMDA receptors, was observed immediately after exposing the hippocampal network to OGD and was inhibited by TTX. Furthermore demonstrating the dependence of Clca1 induction on extrasynaptic NMDA receptors, Clca1 mRNA levels were repressed after

infection of hippocampal neurons with rAAV DN-GIPC, which eliminates the interaction of the adaptor protein GIPC with the extrasynaptic NR2B subunit. Cells pre-treated with this dominant negative form of GIPC were more resistant to ischemia.

This study may provide a new marker for hypoxic/ ischemic conditions: Clca1 is delicately induced by ischemia, in a defined kinetic action and independent of the various brain tissues. It is regulated in an extraordinary manner, by extrasynaptic NMDA receptors. Further examinations of the NR2B subunit triggered pathways, may also lead to targets for therapeutic interventions. The combination of both, a precise diagnostic marker for ischemia and the enhancement of survival mechanisms in neurons, may improve the therapeutic frame and the clinical outcome of patients.