EXTENDED ROLE OF NECROTIC CELL DEATH AFTER HYPOXIA-ISCHEMIA INDUCED NEURODEGENERATION IN THE NEONATAL RAT

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The relative contribution of apoptosis and necrosis after hypoxia-ischemia (HI) in the developing brain is still a matter of debate. We determined the time-course of necrotic cell death in a model of HI in 7-day-old rats and its relationship to caspase-3 activation and apoptotic cell death. Disrupted plasma membrane integrity of necrotic cells was evaluated by "in vivo" intracerebroventricular injection of propidium iodide (PI). PI-positive cells were found in the damaged but not in the contralateral side starting from 30 min after HI in the CA1 region of the hippocampus and increased rapidly in different brain areas. PI co-localized with NeuN but not with GFAP. Both PI and caspase-3 positive cells were found 24h after HI in the injured cerebral cortex, hippocampus and striatum. In the cerebral cortex, at variance with PI labeling, caspase-3 immunoreactivity was present in higher amount in the superficial layers. Caspase-3 and PI showed strong co-localization in the hippocampus, striatum and in the deep layers of the cortex. In contrast, the superficial layers of the cortex showed co-localization only in few cells. At later times, however, cells of the superficial layers were positive to both PI and caspase-3. The time-course of PI labeling and caspase-3 immunoreactivity was similar to that found for calpain and caspase-3 activities. The distribution of caspase-3 positive cells in the injured cerebral cortex was comparable to that found in the TUNEL assay. The superficial layers of the cortex 24h after HI showed many TUNEL-positive cells that mostly displayed apoptotic features. In contrast, in the deep layers of the cortex and in the CA1 and CA2/CA3 region of the hippocampus the number of TUNEL-positive cells was much lower. In these areas, PI-positive cells largely exceed TUNEL-positive cells, and the latter also showed necrotic features. Many PI-positive cells were also cathepsin-B positive and numerous PI/cathepsin-B positive cells expressed activated caspase-3. PI/cathepsin-B labeling was more pronounced in the more severe ischemic regions and increased over time. These data indicate that necrosis has an extended role in the progression of brain injury after neonatal HI and that a different spectrum of suicidal programs can be activated in the same cell. The extended period of caspase-3 activation in PI-positive necrotic cells support the possibility that the apoptotic-to-necrotic continuum found after neonatal HI may ensue as the result of an incomplete execution of the apoptotic program.