The Neuron-Astrocyte-Microglia Triad in a Model of Chronic Cerebral Ischemia in the Rat Hippocampus: Effect of Dipyridamole

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Until recently, neurons were considered to be the basic functional units of the central nervous system while glia cells only to serve as supportive elements. This concept is rapidly changing and it is becoming more and more evident that proper functioning of the neuron-astrocyte-microglia 'triad' is fundamental for the functional organization of the brain (Barres, 2008; Allen and Barres, 2009). The interplay among neurons and glia may be responsible for derangements from normal brain aging to neurodegenerative processes (De Keyser et al., 2008; Sofroniew, 2009). We recently demonstrated, in rat models of normal brain aging and LPS-induced acute inflammation, that astrocytes and microglia actively cooperate in the clearance of apoptotic neurons and neuronal debris, associated with programmed cell death in the hippocampus (Cerbai et al., 2012). Here we studied the interactions between neurons, microglia and astrocytes within the CA1 region of the hippocampus of the rat after bilateral common carotid artery occlusion (bCCAo), a model of chronic cerebral hypoperfusion which leads to persistent ischemic conditions and ultimately to neuronal death. A group of male Wistar rats (n=11) was subjected to permanent bCCAo. A different group of rats, operated for bCCAo, was infused, using an osmotic minipump placed into the jugular vein, with dipyridamole (7 days, 4 mg/kg/day, n=11), an inhibitior of adenosine uptake which increases extracellular adenosine, believed to be an anti-inflammatory agent interacting with the A2 and A3 receptor subtypes. Sham-operated rats were used as controls (n=12). Immunohistochemical staining of neurons, microglia and astrocytes was performed on brain coronal slices 3 months after bCCAo. No differences in astrogliosis, in the number of pyramidal CA1 neurons or in the tickness of the CA1 Str. Pyramidalis was found among the three experimental groups. Neuronal debris present in CA1 Str. Radiatum were significantly more numerous in bCCAo rats in comparison with control rats ($P<0.05$, one way ANOVA and Newman-Keuls). In rats treated with dipyridamole the number of neuronal debris returned to control levels ($P<0.01$, one way ANOVA and Newman-Keuls). In bCCAo rats we found a significant increase in total microglia in comparison to sham operated rats (+18%, $P<0.01$, one way ANOVA and Newman-Keuls) and this effect was completely reverted by dipyridamole ($P<0.01$, one way ANOVA and Newman-Keuls), further substantiating the antiinflammatory effect of this drug. In the CA1 of bCCAo rats we also found many neurons, showing signs of degeneration, closely apposed to and intermingled by astrocyte branches, which appeared to be bisecting the cell body into cellular debris, confirming the results obtained in the previous paper (Cerbai et al., 2012). We found many microglia cells actively phagocytosing the damaged neurons in the CA1 Str. Radiatum of bCCAo rats and, in accordance with our previous paper, these macrophages were in a close interplay with the nearer astrocytes that appear to compartmentalize the neuron that is being engulfed. This finding is consistent with the scavenging activity of microglia upon dying neurons or debris, a possible mechanism that prevents further injury to neighboring neurons. It will be interesting to investigate which intercellular communication mechanisms allow the recruitment and activation of different glial cells in a well-organized reciprocal interaction to scavenge the damaged neurons and to verify the mechanism of the protective effects of dipyridamole found in this chronic cerebral ischemic model.

References

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