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Effects of progesterone on neurite growth inhibitors in the hippocampus following global cerebral ischemia

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ABSTRACT

In this study, the effects of progesterone (P_4) on the immunoreactivity to the neurite growth inhibitor Nogo-A, its receptor (Ng-R), and its effector Rho-A in the rat hippocampus, in association with parameters of spatial learning and memory following global cerebral ischemia, were assessed. Adult male rats were subjected to global cerebral ischemia (15 min), and treated with P_4 or its vehicle at 15 min, 2, 6, 24, 48 and 72 h of reperfusion. Immunoreactivity to Nogo-A, Ng-R, and Rho-A was evaluated at 24 h, 72 h or 7 d, or at 14 d of reperfusion after rats were tested in the Morris Water Maze (MWM). Global cerebral ischemia induced an increase in Nogo-A, Ng-R, and Rho-A immunoreactivities in the cell bodies of CA1 pyramidal neurons at 24 h after global cerebral ischemia, peaking at 72 h, and persisting 14 d later. In addition, at 72 h, a strong immunoreactivity was observed in the hippocampal layers where dendritic arborizations of CA1 pyramidal neurons are located. Treatment with P_4 reduced Nogo-A, Ng-R, and Rho-A immunoreactivities in CA1, particularly at 72 h of reperfusion. These effects of P_4 were consistent with the parameters of a more efficient spatial learning and memory in the MWM, as compared to vehicle-treated rats. Overall results suggest the reduction of neurite growth inhibitory molecules Nogo-A, Ng-R, and Rho-A, as a part of the restorative effects of progesterone possibly allowing the plastic phenomena to occur, able to support the functional preservation of the hippocampus following global cerebral ischemia.

1. Introduction

Transient global cerebral ischemia is a cerebrovascular condition in which the cerebral blood flow is acutely reduced to less than 10%, as occurs in humans during cardiac failure under diverse situations, mostly cardiac arrest (Roine, 1997). It leads to high rates of mortality and permanent disability in survivors, around the world (Karanjia and Geocadin, 2011; Schneider et al., 2009).

Global cerebral ischemia/reperfusion results in a selective neuronal death in particularly vulnerable regions, mainly affecting pyramidal neurons of the CA1 hippocampal subfield

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and layers III and V of the cerebral cortex, spiny neurons of the striatum, and Purkinje cells of the cerebellum (Kirino, 1982; Pulsinelli et al., 1982). In addition, the effects of global cerebral ischemia may include the activation of resident microglia, the production of molecular mediators of both a peripheral and local inflammatory response, and the up-regulation of adhesion molecules in endothelial cells that contribute to the infiltration of leukocytes into the brain (Barone and Kilgore, 2006; Lakhan et al., 2009; Denes et al., 2010). In particular, hippocampal CA1 pyramidal neuron damage is associated both in human beings and experimental animals, with impairment of cognitive functions particularly spatial orientation, learning, and memory (Block, 1999; Hartman et al., 2005; Zola-Morgan et al., 1986).

In the remaining CA1 pyramidal neurons, short- and long-term axonal and dendritic anatomical signs of damage but also of compensatory plastic responses occur after global cerebral ischemia (Briones et al., 2006; Mudrick and Baimbridge, 1989; Neigh et al., 2004; Ruan et al., 2006, 2009, 2012; Skibo and Nikonenko, 2010). Plasticity, however, is limited in part due to the presence of inhibitory components that create a non-permissive environment (Sandvig et al., 2004). Among them, myelin-associated proteins that regulate neurite growth such as Nogo-A are known to restrict anatomical plasticity and functional recovery (Pernet and Schwab, 2012). Nogo-A exerts its effects through the Nogo-receptor (Ng-R), which activates the Rho-A GTPase, mediating neurite growth inhibition (Akbik et al., 2012; Overman and Carmichael, 2013; Pernet and Schwab, 2012). An up-regulation of Nogo-A, Ng-R, and Rho-A, has been demonstrated to occur in pyramidal neurons of the cerebral cortex after global (Zhou et al., 2003), as well as focal (Cheatwood et al., 2008; Jiang et al., 2009) cerebral ischemia. Its inhibition with specific antibodies anti-Nogo-A (IN-1) and anti-Ng-R (NEP1-40), has been demonstrated to promote plastic changes including increases of dendritic arborizations and spine density (Papadopoulos et al., 2002, 2006; Seymour et al., 2005), as well as functional recovery after middle cerebral artery occlusion (MCAO) (Cheatwood et al., 2008; Fang et al., 2010; Lee et al., 2004; Tsai et al., 2007, 2011; Wiessner et al., 2003). In this respect, there are two ongoing studies with Nogo-A antibodies phase I/II clinical trials for patients with spinal cord injury (http://www.clinicaltrial.gov/ct2/show/NCT00406016) and with amyotrophic lateral sclerosis (http://www.clinicaltrial.gov/ct2/show/NCT00875446).

However, the increase of neurite growth inhibitory proteins and the effect of neuroprotective strategies on their expression have not been previously evaluated in the hippocampus after global cerebral ischemia.

Progesterone (P_4) exerts neuroprotective and neurorestorative effects in several models of brain injury (Gibson et al., 2009; Sayeed and Stein, 2009; Schumacher et al., 2012). In particular, when P_4 is administered at early stages of reperfusion after global cerebral ischemia, only a low percentage (20–40% as compared to control rats) of CA1 pyramidal neurons is preserved (Espinosa-García et al., 2013; Moralí et al., 2005, 2011), but cognitive hippocampal-dependent functions are comparable to those of control, non-ischemic rats (Moralí et al., 2011). In addition, CA1 pyramidal neurons of P_4-treated animals show similar cytoarchitectonic characteristics to those of intact rats, in terms of dendritic arborizations and spine density, but with a compensatory increase in the proportion of mushroom-type dendritic spines, conditions not shown by ischemic non-treated rats (Moralí et al., 2012). These findings could be interpreted as progesterone modifying the presence of inhibitory components that constitute part of the physical or chemical barriers to neurite outgrowth or remodeling, therefore creating a permissive environment allowing structural and synaptic plastic repair phenomena to occur after ischemic damage.

The aim of the present study was to assess the effects of progesterone on the immunoreactivity to Nogo-A, Ng-R, and Rho-A in the hippocampus of rats subjected to global cerebral ischemia/reperfusion, in relation to their performance of a spatial learning and memory task.

2. Results

2.1. *Effects of progesterone on spatial learning and memory impairments induced by ischemia*

2.1.1. *Spatial learning*

In the Sham group, escape latencies showed a progressive reduction over the successive trials (Fig. 1a), the values being significantly lower ($p<0.05$) from the third to the sixth day of testing in comparison to those on the first day.

ISCH+VEH rats only showed a slight, non significant reduction of the escape latencies on the successive days of testing as compared to values on the first day. Their escape latencies on days 4–6 were significantly longer ($p<0.01$) than those of the Sham group (Fig. 1a).

On the other hand, rats in the ISCH+P_4 group showed a reduction of the escape latencies in the successive days of testing, similar as those of the Sham group, the values being significantly lower ($p<0.05$) on days 3–6 as compared to the first day (Fig. 1a). Values of escape latencies were significantly lower than those in the ISCH+VEH group on days 4–6 ($p<0.01$, $p<0.05$, and $p<0.001$, respectively).

2.1.2. *Spatial memory*

Number of crossings over the former platform location during the probe trial, was lower in the ISCH+VEH group ($p<0.01$) in relation to that in the Sham group; rats in the ISCH+P_4 group had a significantly higher number of crossings ($p<0.05$) than rats in the ISCH+VEH group, not differing from that of the Sham group (Fig. 1b). This parameter is illustrated in Fig. 1c, where the persistent search for the platform through the target quadrant during the probe trial is displayed by rats in the Sham group. Those in the ISCH+VEH group showed a random pattern, sometimes with circular swimming through the four quadrants and occasionally crossing over the platform location. Rats in the ISCH+P_4 group also showed a persistent search for the platform in the target quadrant, as those in the Sham group (Fig. 1c).

2.2. *Effects of global cerebral ischemia and of progesterone treatment on immunoreactivity to Nogo-A, Ng-R, and Rho-A in the hippocampus*

In the Sham group, Nogo-A (Fig. 2a) and Ng-R (Fig. 3a) immunoreactivities were observed in the *strata radiatum* and