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Acute stress exposure preceding transient global brain ischemia exacerbates the decrease in cortical remodeling potential in the rat retrosplenial cortex

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ABSTRACT

Doublecortin (DCX)-immunoreactive (-ir) cells are candidates that play key roles in adult cortical remodeling. We have previously reported that DCX-ir cells decrease after stress exposure or global brain ischemia (GBI) in the cingulate cortex (Cg) of rats. Herein, we investigate whether the decrease in DCX-ir cells is exacerbated after GBI due to acute stress exposure preconditioning. Twenty rats were divided into 3 groups: acute stress exposure before GBI (Group P), non-stress exposure before GBI (Group G), and controls (Group C). Acute stress or GBI was induced by a forced swim paradigm or by transient bilateral common carotid artery occlusion, respectively. DCX-ir cells were investigated in the anterior cingulate cortex (ACC) and retrosplenial cortex (RS). The number of DCX-ir cells per unit area (mm^2) decreased after GBI with or without stress preconditioning in the ACC and in the RS (ANOVA followed by a Tukey-type test, $P < 0.001$). Moreover, compared to Group G, the number in Group P decreased significantly in RS ($P < 0.05$), though not significantly in ACC. Many of the DCX-ir cells were co-localized with the GABAergic neuronal marker parvalbumin. The present study indicates that cortical remodeling potential of GABAergic neurons of Cg decreases after GBI, and moreover, the ratio of the decrease is exacerbated by acute stress preconditioning in the RS.

1. Introduction

A cerebral remodeling is induced after certain types of brain damage. Certain immunopositive types of neurons apparently contribute to a cortical remodeling in the mammalian brain. These specific neurons include doublecortin (DCX) positive cells (Xiong et al., 2008; Cai et al., 2009), polysialylated-neural cell adhesion molecule (PSA-NCAM) positive cells (Varea et al., 2005), α-internexin positive cells (Blizzard et al., 2011) and Nogo-A positive cells (Cheatwood et al., 2008). These cells are involved in cytoskeletal changes including neurite outgrowth and synaptogenesis and are considered to be potential candidates to induce an effective cortical remodeling.

Remodeling-associated neurons include GABAergic interneurons, especially DCX (Luzzati et al., 2009) and PSA-NCAM positive cells (Castillo-Gómez et al., 2011). Some DCX-immunoreactive (-ir) cells and PSA-NCAM-ir cells are co-localized with the GABAergic neuronal marker parvalbumin (PV) as well as somatostatin (SOM), neuropeptide Y and calbindin (CB) (Varea et al., 2005; Luzzati et al., 2009). Thus far, DCX-ir cells are reported to co-localize with PV, indicating that many of the DCX-ir cells are fast spiking interneurons and have association with neuronal circuit modulation (Kawaguchi and Kondo, 2002).

In rats, DCX-ir cells are detected in the cingulate cortex (Cg), which includes the anterior cingulate cortex (ACC) and the retrosplenial cortex (RS) (Luzzati et al., 2009). The Cg contributes to

Abbreviations: BrdU, bromodeoxyuridine; CB, calbindin; CR, calretinin; CCK, cholecystokinin; Cg, cingulate cortex; DAB, diaminobenzidine; DCX, doublecortin; FJB, Fluorojade B; GBI, global brain ischemia; PV, parvalbumin; PBS, phosphate buffer solution; PSA-NCAM, polysialylated-neural cell adhesion molecule; RS, retrosplenial cortex; SOM, somatostatin; TBS, tris-buffered saline; TBS/TX, triton X-100/TBS solution; VIP, vasoactive intestinal polypeptide.

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emotion, cognition, learning and memory whereby the synaptic remodeling and circuit reconstruction in the Cg play important roles in humans (Degos et al., 1993; Maguire, 2001; Klimecki et al., 2012; Kim et al., 2009) and in rats (Vogt and Miller, 1983; Chen et al., 1994).

A certain enzyme included in the signaling pathway or neural activity in the Cg is altered after stress exposure or brain ischemia in rats (Kurihara et al., 2004) and in humans (Paradiso et al., 2011; Schlösser et al., 2008), while stress exposure or brain ischemia induces the psychiatric and behavioral deficit associated with the role of the Cg in rats (Naudon and Jay, 2005; Bertaina-Anglade et al., 2006; Bendel et al., 2005; Caldwell et al., 1997) and in humans (Bassetti et al., 1996; Alexander, 1997; Roozendaal, 2002; Oomen et al., 2010). However, it is still not clear what the underlying cellular mechanisms are for stress and/or brain ischemia as they relate to remodeling in the Cg.

We previously researched and published findings regarding the decrease in DCX expression in the RS after acute stress exposure (Kutsuna et al., 2012) and findings regarding the same after transient global brain ischemia (GBI) (Kutsuna et al., 2013: proceedings). Furthermore, we reported that preconditioning with acute stress exposure exacerbates a decrease in DCX expression after GBI in the RS (Kutsuna et al., 2014: proceedings). The present study uses data from previous proceedings and incorporates new data regarding the ACC as they relate to cellular mechanisms underlying acute stress exposure and GBI.

2. Materials and methods

2.1. Animals, preconditioning with acute stress exposure, transient GBI

Twenty male Sprague-Dawley rats (body weight 250–300 g) were used for this study. The rats were divided into 3 groups: acute stress exposure preceding GBI (stress → GBI, $N = 6$, Group P), non-stress exposure, thus normal conditions preceding GBI (normal condition → GBI, $N = 6$, Group G), and controls (normal condition → sham operation, $N = 8$, Group C). The rats were purchased from Charles River Laboratories (Saitama, Japan) and bred at the animal housing facility of Nihon University School of Medicine. The colony was maintained at 22–23 °C on a 12 h light/dark cycle (lights on at 08:00 h). All experimental procedures were designed in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, National Academy Press, Washington, DC, USA; 2003) and approved by the Animal Care and Use Committee of Nihon University.

Preconditioning with acute stress exposure was induced by the forced swim paradigm based on previous protocols (Porsolt et al., 1978). Day 1: In brief, the rats were forced to swim individually in a water tank (22.5 cm diameter, 45.5 cm height, 15 cm water depth) at 25 °C. They were removed after a 15-min induction period in the water and towel dried before being returned to their home cages. Day 2: The rats were returned to the tank for a second test of 5-min duration 24 h after the first immersion. The primary endpoint of this induction is immobility. Immobility means floating in the water without struggling and making only movement necessary to keep the head above the water (Detke et al., 1995). The mean time of immobility on day 2 was 210 ± 45.26 (described as s ± SD). The mean count of immobility was 31 ± 6.53 (described as times ± SD). The time and count of immobility were considered sufficient stress according to previous results (Hemby et al., 1997; Lucki, 1997; Overstreet et al., 2010).

Day 3: Transient GBI was induced by a bilateral common carotid arterial occlusion for 10 min to partially modify previous reports (Yagita et al., 2001). The rats were anesthetized with pentobarbital (Somnopentyl, Kyoritsu Seiyaku, Japan; 15 mg/kg body weight i.p.) and 3% isoflurane in a 70% N_2O/30% O_2 solution and then maintained on 1.5% isoflurane during surgery. Both vertebral arteries were coagulated and cut through each alar foramen with a needle. Both common carotid arteries were occluded with titanium aneurysmal clips for 10 min. Rats that showed respiratory distress were excluded. Rectal temperature was continuously monitored during ischemia and was maintained at 37 °C.

The rats were anesthetized with pentobarbital (30 mg/kg body weight i.p.) on day 7 after the GBI and were perfusion-fixed transcardially with lactated Ringer's solution, followed by perfusion with 4% paraformaldehyde in a 0.1 M phosphate buffer solution (PBS) (pH 7.4). The brains were removed from the skull. Coronal sections (50 μm) were cut on a vibratome (Vibratome Series 1000 Sectioning System, Technical Products International Inc., St. Louis, Missouri, USA), and the sections were stored at 4 °C in PBS (pH 7.4). We identified the ACC and the RS of the Cg by referring to the rat atlas (Paxinos and Watson, 2008).

2.2. Immunohistochemistry, fluorescent immunohistochemistry, analysis

Free-floating sections were placed in a 0.9% H_2O_2 solution for 5 min. The sections were then washed once in Tris-buffered saline (TBS) (pH 7.4) for 15 min followed by two consecutive 15 min washes in a 1% Triton X-100/TBS solution (TBS/TX) (pH 7.4). Anti-DCX antibodies (polyclonal goat, 1:2000, Santa Cruz Biotechnology, California, USA) were diluted in TBS/TX containing 3% normal rabbit serum. The sections were incubated with primary antibodies at 4 °C for 24 h, and rinsed once in TBS/TX for 15 min followed by two consecutive 15 min washes in TBS.

The secondary antibodies were biotin-labeled rabbit anti-goat IgG antibodies diluted in TBS/TX containing 1% normal rabbit serum (1:400, Vector Laboratories, Burlingame, California, USA). The sections were agitated with secondary antibodies at room temperature for 2 h followed by one 15 min wash in TBS/TX and two 15 min washes in TBS.

The sections were incubated with ABC solution (1:200, Vectastain ABC Elite kit, Vector Laboratories) in TBS/TX at room temperature for 1 h, followed by one 15 min wash in TBS/TX and two 15 min washes in TBS. For color development, the sections were incubated with 0.005% 3,3′-diaminobenzidine (DAB), 3% H_2O_2, and 0.4% $NiCl_2$ in TBS at room temperature for 1–5 min, which yielded a brownish color. This was followed by three 15 min washes in TBS. The sections were placed on gelatin-coated slides, dehydrated in ethanol, cleared in xylene, and mounted with coverslips using Mount-Quick (Daido Sangyo, Tokyo, Japan).

Double staining by fluorescent immunohistochemistry was performed, and a preliminary treatment was undertaken with 100% methanol for 5 min. The primary antibodies and secondary antibodies used for this study are listed in Table 1. The sections were agitated with secondary antibodies at room temperature in a dark room for 2 h, followed by six 15 min washes with TBS. Fluorescent double staining was performed as described above, employing an avidin/biotin blocking kit (Vector) after the first secondary antibody sensitization.

We used Biozero (BZ-8000; KEYENCE, Osaka, Japan) and a BZ-Analyzer (KEYENCE) to prepare the microphotographs and employed a VH-Analyzer (KEYENCE) to count the number of immunostain-positive cells or Fluorojade B (FJB)-positive cells. NEUROLUCIDA (Version 3; MicroBrightField, Williston, Vermont, USA) was used to analyze the area and all cell counts without double counting of positive cells in the ACC (bregma anterior, 1.20 and 0.70 mm) and the RS (bregma posterior, 2.80 and 3.30 mm). The immunopositivity of DCX on DAB-developed sections was judged