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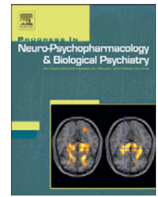

Differential inhibition of neurogenesis and angiogenesis by corticosterone in rats stimulated with electroconvulsive seizures

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ABSTRACT

Antidepressant drugs and electroconvulsive seizure (ECS)-treatment, an animal model of electroconvulsive therapy, induce neurogenesis in adult rats. Stress and high levels of corticosterone (CORT) on the contrary inhibit neurogenesis. Hippocampal neurogenesis has been described to occur in an angiogenic niche where proliferation of neural progenitors takes place in an environment with active vascular growth. Here we investigate the effect of ECS-treatment on the proliferation of endothelial cells and neuronal precursors in hippocampus of CORT-treated rats. Bromodeoxyuridine (BrdU) was used to identify dividing cells. The number of newborn neuronal precursors and endothelial cells was quantified in the subgranular zone (SGZ) and the molecular layer (ML) of the dentate gyrus. The increase in neuronal precursor proliferation in the SGZ following ECS-treatment was not inhibited by elevated levels of CORT despite CORT strongly inhibiting ECS-induced endothelial cell proliferation. Also in the ML CORT-treatment inhibited the ECS-induced angiogenic response. We conclude that despite common factors regulating neurogenesis and angiogenesis, ECS-induced proliferation of neuronal precursors can take place even if the angiogenic response is blunted. Whether inhibition of angiogenesis affects other steps in the chain of events leading to the formation of fully integrated granule neurons remains to be elucidated.

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1. Introduction

Antidepressant treatment stimulates hippocampal neurogenesis (Madsen et al., 2000; Scott et al., 2000; Malberg et al., 2000) in adult mammals, including primates (Pereira et al., 2007). Although the implication of enhanced neurogenesis is not known, it has been proposed to be a prerequisite for the antidepressant effect of fluoxetine (Santarelli et al., 2003). Similarly, angiogenesis, the growth of new vessels, is also stimulated by antidepressant treatment, including electroconvulsive seizures (ECS), the animal analogue to electroconvulsive therapy, in the hippocampus (Hellsten et al., 2004; Warner-Schmidt and Duman, 2007) the hypothalamus (Jansson et al., 2006) and the prefrontal cortex (Madsen et al., 2005; Kodama et al., 2005; Czeh et al., 2007).

Contrary to antidepressants, stress and glucocorticoids decrease neurogenesis (Gould et al., 1997; Czeh et al., 2001; Hellsten et al., 2002) and this has been proposed to underlie the symptoms as well as the pathophysiology of mood disorders (Duman, 2004). Stress also decreases proliferation of endothelial cells in the prefrontal cortex (Banasr et al., 2007). In the hippocampus chronic stress has been shown to inhibit the proliferation of vascular-associated cells stronger than cells not associated with vessels (Heine et al., 2005). This is in line with our own data that CORT exerts a strong inhibition on the proliferation of endothelial cells (unpublished data).

In adult mammals neurogenesis is mainly taking place in two discrete brain regions, the subgranular zone (SGZ) of the hippocampus (Altman and Das, 1965; Eriksson et al., 1998) and the subventricular zone (SVZ), lining the lateral ventricles (Doetsch et al., 1997). In SGZ clusters of neuronal precursor cells, expressing various neuronal markers (e.g. doublecortin (DCX)) (des Portes et al., 1998), are found in close proximity to proliferating endothelial cells expressing the endothelial cell marker rat endothelial cell antigen-1 (RECA-1) (Duijvestijn et al., 1992). Adult hippocampal neurogenesis has, because of this micro-anatomical organization, been described as occurring in an angiogenic niche (Palmer et al., 2000). The apparent co-regulation of angiogenesis and neurogenesis in SGZ is supported by the fact that a number of growth factors stimulate both neurogenesis and angiogenesis, including fibroblast growth factor-2 (Ghosh and Greenberg, 1995), vascular endothelial growth factor (VEGF) (Jin et al., 2002), brain-derived neurotrophic factor (BDNF) (Kim et al., 2004) and neuropeptide Y (Howell et al., 2005; Zukowska-Grojec et al., 1998).

Given our previous finding that neuronal progenitors and endothelial cells proliferate in response to ECS-treatment in a seemingly coordinated fashion (Hellsten et al., 2004), and the inhibitory effect of CORT on both

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BrdU, bromodeoxyuridine; CORT, corticosterone; DCX, doublecortin; ECS, electroconvulsive seizures; KPBS, potassium phosphate-buffered saline; ML, molecular layer; *n*, number of animals; NDS, normal donkey serum; PBS, phosphate phosphate-buffered saline; RECA-1, rat endothelial cell antigen 1; RT, room temperature; SGZ, subgranular zone; SVZ, subventricular zone; VEGF, vascular endothelial growth factor.

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endothelial and neuronal cell proliferation, we wanted to investigate how CORT-treatment affects the angiogenic niche for adult neurogenesis.

2. Materials and methods

2.1. Animals and treatment

Adult male Wistar rats (Møllegaard, Denmark), weighing 200 g at the start of the experiment, housed three per cage, on a 12-h light dark cycle with unlimited access to food and water, were used. Experimental procedures were carried out according to the guidelines set by the Malmö-Lund Ethical Committee for the use and care of laboratory animals. The rats (n=22) were divided into the following groups: (1) seven days of CORT-injections (Sham+CORT, n=5); (2) seven days of CORT-injections and one daily ECS-treatment the first five days (ECS+CORT, n=6); (3) seven days of vehicle injections (Sham+Vehicle, n=6); (4) seven days of vehicle injections and one daily ECS-treatment the first five days (ECS+Vehicle, n=5) (Fig. 1). The weight of the rats was continuously monitored during the experiment.

2.2. Administration of electroconvulsive seizures

During the first five days, all rats were subjected either to a daily ECS- or sham-treatment at 10.00. ECS-treatment was delivered via ear clips (Somedic sales AB, Sweden) (50 mA, 0.5 s and 50 Hz unidirectional square wave pulses).

The rats were monitored to ensure that clonic movements of the face and forelimbs occurred after ECS for 20–30 s (indicative of motor limbic seizures). Sham-treated rats were handled identically to the ECS-treated rats except that no current was passed.

2.3. Administration of corticosterone

A stock emulsion of CORT, solubilized in sesame oil was injected subcutaneously in the neck region (40 mg/kg) every 24 h at 9.00. This dose is sufficient to elevate blood levels of CORT over a 24 h-period (Sapolsky et al., 1985). Control rats received sesame oil injections.

2.4. Administration of BrdU

Bromodeoxyuridine (B5002, Sigma-Aldrich, St Louis, MO, USA) was dissolved in phosphate-buffered saline (PBS) and administered intraperitoneally. All rats received BrdU-injections (50 mg/kg) twice daily, in 12-h intervals (8.00 and 20.00) during the last five days of the experiments.

2.5. Tissue preparation

Twelve hours after the last BrdU-injection, rats were anesthetized with sodium pentobarbital and, in the absence of nociceptive reflexes, transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). Following decapitation, the brain was removed from the skull and postfixed at 4 °C overnight, followed by cryoprotection in 30% sucrose in PBS. Coronal 40 µm sections were cut through the mid-dorsal hippocampus (−3.30 mm to −4.52 mm relative to bregma) (Paxinos and Watson, 1986), defined as extending from the point when the dorsal and ventral blade of the dorsal GCL fuses in a tip (−3.30 mm) to where the pyramidal cell layer of the dorsal and ventral hippocampus merges (−4.52 mm). Sections were stored in antifreeze cryoprotectant solution at −20 °C until the immunohistochemical procedure.

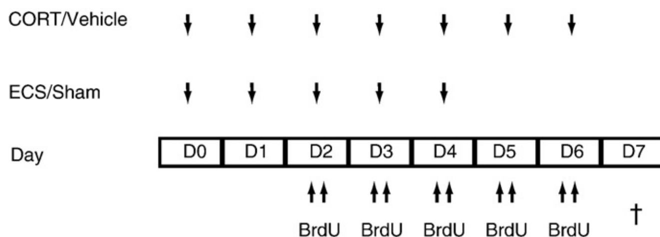

Fig. 1. Experimental design and group assignment. Rats were divided into four treatment groups: vehicle; corticosterone (CORT); vehicle+5 electroconvulsive seizures (ECS); CORT+5ECS. Vehicle- or CORT-injections were given once daily on day 0 to day 6. Sham- or ECS-treatment was given once daily for five days (day 0 to day 4). All rats received two daily bromodeoxyuridine (BrdU)-injections on day 2 to day 6 and were transcardially perfused on day 7, 12 h after the last BrdU-injection.

2.6. Staining procedures

2.6.1. BrdU/rat endothelial cell antigen-1-staining

Sections were rinsed in potassium phosphate buffered saline (KPBS), pre-incubated in blocking solution (KPBS with 0.25% Triton X-100 (KPBS+)) and 5% normal donkey serum (NDS) for 1 h at room temperature (RT) followed by incubation with 1:100 mouse anti-RECA-1 (Serotec MCA 970, Oxford, UK) in blocking solution for 48 h at 4 °C. Sections were rinsed with KPBS+, incubated with 1:200 biotinylated donkey anti-mouse (Jackson 715-065-151, Jackson Immuno Research, West Grove, PA) in blocking solution, for 24 h at 4 °C. Sections were then rinsed in KPBS+ before incubation with 1:200 Alexa 488-conjugated streptavidine (Molecular Probes, Eugene, OR) in KPBS+ for 24 h in darkness, at 4 °C. After rinsing in KPBS, sections were fixed in 4% PFA for 10 min at RT, subsequently rinsed in KPBS and then incubated in 1 M hydrochloric acid at 65 °C for 30 min. After rinsing sections were pre-incubated in blocking solution for 1 h in darkness at RT, then incubated with 1:100 rat anti-BrdU (Oxford Biotechnology, OBT 0030, Kidlington, UK) in blocking solution for 40 h in darkness at 4 °C. Sections were rinsed twice in KPBS+ and twice in KPBS+, 2% NDS (modified blocking solution) before incubation with 1:200 Cy-3-conjugated donkey anti-rat (Jackson 712-165-153, Jackson Immuno Research, West Grove, PA) in modified blocking solution for 24 h in darkness at 4 °C. After rinsing, sections were slide mounted, air dried, and coverslipped.

2.6.2. BrdU/doublecortin-staining

Sections were rinsed in KPBS and then incubated in 1 M hydrochloric acid at 65 °C for 30 min, followed by rinsing in KPBS and subsequent pre-incubation in blocking solution. Sections were then incubated with 1:100 rat anti-BrdU and 1:100 goat anti-doublecortin (DCX) (sc-8066, Santa Cruz Biotechnology, Santa Cruz, CA) in blocking solution for 40 h in darkness at 4 °C. Sections were rinsed in blocking solution and then incubated with 1:200 Alexa 488-conjugated donkey anti-goat (Molecular Probes, Eugene, OR) and 1:200 Cy-3-conjugated donkey anti-rat in blocking solution for 24 h in darkness at 4 °C. After rinsing, sections were slide mounted, air dried, and coverslipped.

2.7. Data quantification

Cell proliferation was assessed in the SGZ and ML of the dentate gyrus (DG), using an Olympus fluorescence microscope (Olympus Optical Co. Ltd., Tokyo, Japan) with a 40× objective. Cells lying within two cell diameters of the granule cell and hilar border were included in the SGZ count. Proliferated endothelial cells were counted in all vessel types in the SGZ and ML (as previously described in Hellsten et al., 2004). For cells in the SGZ no distinction was made between endothelial cells and neural precursors. Every sixth section throughout the mid-dorsal hippocampus (resulting in an average of five sections per animal) was counted and cell counts were averaged and expressed as means per section.

A cell cluster was (in accordance with Palmer et al., 2000) defined as any two or more cells within 25 µm of each other in the same

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