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SCIENCE DIRECT

Experimental Neurology 195 (2005) 71 – 80

Experimental Neurology

www.elsevier.com/locate/yexnr

Regular Article

Quantitative analysis of the generation of different striatal neuronal subtypes in the adult brain following excitotoxic injury

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Received 26 November 2004; revised 22 February 2005; accepted 30 March 2005
Available online 2 June 2005

Abstract

Recent findings in adult rodents have provided evidence for the formation of new striatal neurons from subventricular zone (SVZ) precursors following stroke. Little is known about which factors determine the magnitude of striatal neurogenesis in the damaged brain. Here we studied striatal neurogenesis following an excitotoxic lesion to the adult rat striatum induced by intrastriatal quinolinic acid (QA) infusion. New cells were labeled with the thymidine-analogue 5-bromo-2′-deoxyuridine (BrdU) and their identity was determined immunocytochemically with various phenotypic markers. The unilateral lesion gave rise to increased cell proliferation mainly in the ipsilateral SVZ. At 2 weeks following the insult, there was a pronounced increase of the number of new neurons co-expressing BrdU and a marker of migrating neuroblasts, doublecortin, in the ipsilateral striatum, particularly its non-damaged medial parts. About 80% of the new neurons survived up to 6 weeks, when they expressed the mature neuronal marker NeuN and were preferentially located in the outer parts of the damaged area. Lesion-generated neurons expressed phenotypic markers of striatal medium spiny neurons (DARPP-32) and interneurons (parvalbumin or neuropeptide Y). The magnitude of neurogenesis correlated to the size of the striatal damage. Our data show for the first time that an excitotoxic lesion to the striatum can trigger the formation of new striatal neurons with phenotypes of both projection neurons and interneurons.

Keywords: Neurogenesis; Excitotoxic injury; Rat striatum

Introduction

In the intact adult brain, the neural stem/precursor cells in the subventricular zone (SVZ), lining the lateral ventricle, generate new interneurons which migrate to the olfactory bulb (Altman, 1969; Lois and Alvarez-Buylla, 1994). Recent findings in rodents have provided evidence that the SVZ precursors can be recruited following stroke induced by transient middle cerebral artery occlusion (MCAO), which causes extensive damage to striatum and overlying parietal cortex. Stroke was found to trigger increased neural proliferation in the SVZ (Jin et al., 2001; Zhang et al., 2001). The new immature neurons migrate into the damaged striatum, where a substantial portion of them express markers of striatal medium spiny projection neurons (Arvidsson et al., 2002; Jin et al., 2003; Parent et al., 2002). Thus, the new neurons seem to develop the phenotype of most neurons destroyed by the ischemic lesion. In addition, intraventricular infusion of epidermal growth factor after stroke in mice has been reported to generate cells expressing

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo-2′-deoxyuridine; ChAT, choline acetyltransferase; DARPP-32, dopamine- and adenosine 3′5′-monophosphate-regulated phosphoprotein with a molecular weight of 32 kDa; Dcx, doublecortin; GABA, γ-aminobutyric acid; KPBS, potassium phosphate-buffered saline; MCAO, middle cerebral artery occlusion; NeuN, neuron-specific nuclear antigen; NPY, neuropeptide Y; PARV, parvalbumin; PBS, phosphate-buffered saline; PFA, paraformaldehyde; QA, quinolinic acid; SVZ, subventricular zone.

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0014-4886/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2005.03.017

parvalbumin, i.e., a marker of a specific type of striatal interneuron (Teramoto et al., 2003).

The findings that following a stroke, new neurons can be formed in damaged regions in which neurogenesis does not normally occur, e.g., striatum, have raised the possibility that such self-repair could become of therapeutic value (Abrahams et al., 2004). However, it is unclear whether the new neurons are functional and if they become integrated into existing neural and synaptic networks. Furthermore, the stroke-induced neurogenic response may be ineffective because the majority of the new neurons die during the first weeks after they have been formed (Arvidsson et al., 2002). In order to be able to promote self-repair in the brain, it is necessary to identify those mechanisms which regulate the formation of new neurons. Currently, very little is known about which factors determine the occurrence and magnitude of adult striatal neurogenesis. Here, we have studied striatal neurogenesis following an excitotoxic lesion to the adult rat striatum, produced by intrastriatal quinolinic acid (QA) infusion. The objectives were two-fold: First, to explore whether injury to the striatum caused by a non-ischemic insult can induce the generation of new striatal neurons with the phenotypes of projection neurons and interneurons; second, to determine if the extent of injury influences the various steps of striatal neurogenesis, and what is the location of the new neuroblasts and mature neurons in relation to the damage in the striatum.

Materials and methods

Animals

Male Wistar rats (n = 23; body weight 530–580 g) were obtained from Charles River (Germany). The animals were housed under 12:12-h light/dark conditions with ad libitum access to food and water. Experimental procedures were conducted according to the guidelines set by the Malmö-Lund Ethical Committee for the use and care of laboratory animals.

Quinolinic acid and BrdU administration

Rats were anaesthetized with sodium pentobarbital (60 mg/kg ip) and infused with QA (2 μl (225 nmol dissolved in phosphate-buffered saline (PBS)) during 2 min; the Hamilton syringe was withdrawn after 5 min) into the right striatum at the following coordinates: 1.2 mm rostral and 3.2 mm lateral to bregma and 5.0 mm ventral from dura, tooth-bar at 0 mm (Paxinos and Watson, 1997). Rats subjected to sham surgery were infused with saline. Intraperitoneal injections of BrdU (5-bromo-2′-deoxyuridine, 50 mg/kg body weight, Sigma-Aldrich, St Louis; dissolved in PBS) were given twice daily during 2 weeks starting on the day after surgery. A group of intact rats, not subjected to any surgeries, were injected similarly. Animals were allowed to survive 1 day (six QA-infused, six saline-infused and six intact animals) or 4 weeks (five QA-infused animals) after the last BrdU injection.

Immunohistochemistry

Animals were deeply anaesthetized with sodium pentobarbital and transcardially perfused with saline followed by ice-cold formaldehyde solution (4% paraformaldehyde (PFA) in 0.1 M PBS, pH 7.4). Brains were removed and postfixed in the same solution overnight. The PFA-solution was then replaced by 20% sucrose in 0.1 M PBS for at least 24 h for cryoprotection. The brains were sectioned on a freezing microtome at 30 μm thickness. Sections were stored at −20°C in cryo-protective solution.

Free-floating sections were denatured by incubation in 1 M hydrochloric acid for 30 min at 65°C (except for staining including the anti-choline acetyltransferase (ChAT); 1 M hydrochloric acid for 10 min at 65°C followed by 20 min at room temperature). Following rinsing with potassium phosphate-buffered saline (KPBS), sections were preincubated for 1 h in 5% of appropriate sera (normal donkey serum for BrdU, normal horse serum for doublecortin (Dcx), neuron-specific nuclear antigen (NeuN), dopamine- and adenosine 3′:5′-monophosphate-regulated phosphoprotein with a molecular weight of 32 kDa (DARPP-32), parvalbumin (PARV) or ChAT, and normal goat serum for neuropeptide Y (NPY)) in KPBS containing 0.25% Triton-X. Sections were then incubated with rat anti-BrdU (1:100, Oxford Biotechnology Ltd, Oxfordshire, UK) and either of the following primary antibodies against phenotypical markers; goat anti-Dcx (1:400, Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-NeuN (1:100, Chemicon International, Temecula, CA), mouse anti-DARPP-32 (1:20000, provided by P. Greengard), mouse anti-PARV (1:2000, Sigma), rabbit anti-NPY (1:1500, Sigma), or mouse anti-ChAT (1:750, Chemicon International, Temecula, CA) in pre-incubation solution for 36 h at 4°C. Sections were then rinsed with KPBS containing 0.25% Triton-X, followed by 2 h incubation with Cy3-conjugated donkey anti-rat secondary antibody (1:200, Jackson ImmunoResearch Laboratories Inc, West Grove, PA) for visualization of BrdU together with either biotinylated horse anti-goat (for Dcx), horse anti-mouse (for NeuN, DARPP-32, PARV, and ChAT), or goat anti-rabbit (for NPY) (1:200, Vector Laboratories, Burlingame, CA) at room temperature in darkness. Following rinsing, sections were finally incubated with Alexa 488-conjugated streptavidin (1:200, Molecular Probes Inc, Eugene, OR) in KPBS for 2 h, at room temperature in darkness. The sections were mounted onto glass slides. After drying, they were rinsed in distilled water and dried again before being cover-slipped with PVA-DABCO mounting medium.

Microscopical analysis

All analyses were conducted by observers blind to treatment conditions. BrdU+ cells in the SVZ were counted