Behavioural Brain Research 316 (2017) 145–151

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

ChAT-positive neurons participate in subventricular zone neurogenesis after middle cerebral artery occlusion in mice

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HIGHLIGHTS

- SVZ choline acetyltransferase (ChAT)$^+$ neurons promote neurogenesis in adult mice.
- SVZ neurogenesis was elevated after middle cerebral artery occlusion (MCAO) in mice.
- The cholinergic system in the SVZ was activated after MACO.
- Post-stroke neurogenesis was enhanced by donepezil and abolished by atropine.
- SVZ ChAT$^+$ neurons may promote neurogenesis via the FGFR signaling pathway.

ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form 30 August 2016
Accepted 3 September 2016
Available online 5 September 2016

Keywords:
ChAT+ neurons
Stroke
Neurogenesis
Subventricular zone

ABSTRACT

The mechanisms of post-stroke neurogenesis in the subventricular zone (SVZ) are unclear. However, neural stem cell-intrinsic and neurogenic niche mechanisms, as well as neurotransmitters, have been shown to play important roles in SVZ neurogenesis. Recently, a previously unknown population of choline acetyltransferase (ChAT)$^+$ neurons residing in rodent SVZ were identified to have direct control over neural stem cell proliferation by indirectly activating fibroblast growth factor receptor (FGFR). This finding revealed possible neuronal control over SVZ neurogenesis. In this study, we assessed whether these ChAT$^+$ neurons also participate in stroke-induced neurogenesis. We used a permanent middle cerebral artery occlusion (MCAO) model produced by transcranial electrocoagulation in mice, atropine (muscarinic cholinergic receptor [mAchR] antagonist), and donepezil (acetylcholinesterase inhibitor) to investigate the role of ChAT$^+$ neurons in stroke-induced neurogenesis. We found that mAchRs, phosphorylated protein kinase C (p-PKC), and p-38 levels in the SVZ were upregulated in mice on day 7 after MCAO. MCAO also significantly increased the number of BrdU/doublecortin-positive cells and protein levels of phosphorylated–neural cell adhesion molecule and mammalian achaete scute homolog-1. FGFR was activated in the SVZ, and doublecortin-positive cells increased in the peri-infarction region. These post-stroke neurogenic effects were enhanced by donepezil and partially decreased by atropine. Neither atropine nor donepezil affected peri-infarct microglial activation or serum concentrations of TNF-α, IFN-γ, or TGF-β on day 7 after MCAO. We conclude that ChAT$^+$ neurons in the SVZ may participate in stroke-induced neurogenesis, suggesting a new mechanism for neurogenesis after stroke.

1. Introduction

Neurogenesis continues throughout the life of adult mammals and has been clearly demonstrated at two locations: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampus [1]. Neural stem cells (NSCs) residing in the SVZ give rise to neuroblasts that migrate to the olfactory bulb in mammals [2]. The fate of such neuroblasts is unknown

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http://dx.doi.org/10.1016/j.bbr.2016.09.007
0166-4328/

because the density of neuroblasts in human SVZ is similar to that in the subgranular zone, but limited postnatal neurogenesis is observed in the human olfactory bulb [3]. Recent research has shown that NSCs in the human SVZ can develop into new neurons that integrate into the striatum [4], suggesting that neurogenesis in the SVZ may be utilized therapeutically in neurologic diseases.

The mechanism of postnatal and adult neurogenesis in the SVZ is unclear. Most research shows that it is controlled by an intrinsic mechanism in which NSCs interact with extracellular and niche-driven cues [1,5,6]. However, recent studies showed that neurotransmitters can also influence NSC proliferation [7,8], indicating possibilities for higher level inputs during SVZ neurogenesis. Recently, Paez-Gonzalez and colleagues [9] identified a previously unknown population of choline acetyltransferase (ChAT)-positive (+) neurons residing in the rodent SVZ neurogenic niche that can exert activity-dependent control over the proliferative activity and neurogenic response of NSCs in the SVZ. They described a new mechanism by which activity of neural networks can be translated into NSC-dependent plasticity. However, when and how this system is used to regulate neurogenesis remains unknown.

Ischemic stroke induces proliferation of NSCs in the SVZ and attracts newborn neurons to the injury zone [10]. Additionally, treatments that increase SVZ neurogenesis have been correlated with enhanced functional recovery [11,12]. However, the mechanism of stroke-induced neurogenesis is not clear. In this study, we investigated whether the newly found $ChAT^{+}$ neurons that reside in the SVZ niche also participate in stroke-induced neurogenesis.

2. Materials and methods

2.1. Animals and ethics statement

Male C57BL/6 mice (25–30 g, 12–14 weeks old) were purchased from the Animal Experimental Center of Zhengzhou University. All mice were housed in plastic cages (8 per cage) with free access to food and water and were maintained at a constant temperature of $22 \pm 1\,^{\circ}C$. All protocols were approved by the Animal Care and Use Committee of Zhengzhou University. All efforts were made to minimize the number of animals used and their suffering.

2.2. Middle cerebral artery occlusion model

Mice were subjected to the permanent middle cerebral artery occlusion (MCAO) model of ischemic stroke by transcranial electrocoagulation as previously described [13]. Briefly, we anesthetized mice with an intraperitoneal injection of 4% chloral hydrate (400 mg/kg) and made a 1-cm skin incision between the left ear and eye. We carefully detached the temporal muscle from the skull by using bipolar electrocoagulation forceps at 12 W. We identified the MCA below the skull in the rostral part of the temporal area, dorsal to the retro-orbital sinus, and thinned out the bone with a drill directly above the MCA branch. The arteries proximal and distal to the bifurcation were coagulated with the bipolar electrocoagulation forceps at 7 W. We then replaced the temporal muscle, sutured the wound, and placed the animal in a nursing box at 32 °C to recover from the anesthesia. The sham procedure was performed as described above without coagulation of the MCA.

2.3. Treatment and groups

We randomly assigned the mice to six groups [14]: sham-operated mice treated with vehicle (Sham + vehicle, $n = 24$), MCAO-operated mice treated with vehicle (MCAO + vehicle, $n = 32$), sham-operated mice treated with atropine (Sham + atropine, $n = 16$), MCAO mice treated with atropine (MCAO + atropine, $n = 24$), sham-operated mice treated with donepezil (Sham + donepezil, $n = 16$), and MCAO mice treated with donepezil (MCAO + donepezil, $n = 24$). Donepezil (selective acetylcholinesterase [AchE] inhibitor, 5 mg/kg/day, Abcam, Cambridge, MA, USA) was dissolved in drinking water and administrated orally on days 1, 3, 5, and 7 after MCAO or sham surgery [15]. Atropine (competitive inhibitor of muscarinic acetylcholine receptors [mAchRs], 5 mg/kg/day, Abcam) was administrated by intragastric delivery on days 1, 3, 5, and 7 after MCAO or sham surgery [15]. All mice except those prepared for neurologic function assessment received intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdU; 50 mg/kg, Sigma-Aldrich, St Louis, MO, USA) once daily for 7 days.

2.4. Neurologic function assessment

We used neurologic deficit scoring and the cylinder test to assess neurologic deficits of the mice as previously described [13,16]. The assessments were conducted on days 1, 7, 14, and 28 after the MCAO procedure ($n = 10$ per group). Neurologic deficits were scored by using a 5-point scale [17]: 0 = no neurologic deficit, 1 = failure to fully extend right forepaw, 2 = circling to the right, 3 = falling to the right, and 4 = no spontaneous walking and depressed level of consciousness. For the cylinder test [13], mice were placed in a transparent acrylic cylinder (diameter: 8 cm, height: 25 cm), and their forelimb activity as they reared up against the wall was recorded. Independent forelimb use was recorded according to the following standards: (1) contact of the cylinder wall with one forelimb during full rear and (2) landing with only one forelimb on the floor after full rear. An investigator blinded to the treatment groups recorded the number of impaired right and non-impaired left forelimb contacts, with a total of 20 contacts for each mouse.

2.5. Infarct volume analysis

On day 7 after MCAO, we sacrificed eight mice per group to measure infarct volume with Nessl staining as previously described [18]. Briefly, after being deeply anesthetized with 10% chloral hydrate, the mice were transcardially perfused with 0.01 mol/L phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde in 0.01 mol/L PBS (pH 7.4). Brains were removed, post-fixed in 4% paraformaldehyde overnight, and stored in 30% sucrose/0.01 mol/L PBS until the tissue sank. Then the brain was serially cut into 20-μm-thick floating sections every 480 μm by cryoultramicrotomy (CM1100, Leica Biosystems, Germany) and stored in antifreeze buffer in 24-well plates at $-20\,^{\circ}C$ for future use. The serial brain sections were placed on slides for Nessl staining according to the standard protocol of the manufacturer (Beyotime Institute of Biotechnology, China). All slides were viewed and photographed under a microscope (Zeiss Stemi 2000-CS, Zeiss, Germany). The infarct volume was analyzed by the Swanson method [19] to correct for edema: Ischemic area = (cortex area of contralateral side) − (non-ischemic cortex area of ipsilateral side).

2.6. Immunofluorescence analysis

The brain sections used for infarct volume analysis were also used for immunofluorescence analysis [14]. Briefly, seven or eight sections of each mouse were washed three times for 5 min in 0.01 mol/L PBS. The sections used for BrdU detection were incubated in 2 mol/L HCl for 30 min followed by 0.1 mol/L sodium borate buffer (pH 8.5) for 10 min. After incubation with 1% bovine serum albumin in PBST (PBS + 0.25% Triton X-100) for 30 min, the sections were incubated with sheep anti-ChAT antibody (1:1000, Abcam), goat anti-doublecortin (DCX) antibody (1:200, Santa Cruz Biotechnology, Dallas, TX, USA), rat anti-BrdU antibody (1:250, Abcam), or rabbit anti-Iba1 antibody (1:500, Abcam) for 1 h at room temperature or overnight at $-4\,^{\circ}C$. Then they were washed three times with