Epilepsy & Behavior 70 (2017) 125–130

Contents lists available at ScienceDirect

Epilepsy & Behavior

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

Transplantation of inhibitory precursor cells from medial ganglionic eminence produces distinct responses in two different models of acute seizure induction

Daisyléa de Souza Paiva [a,1], Simone Amaro Alves Romariz [a,1], Maria Fernanda Valente [a], Luiz Bruno Moraes [a], Luciene Covolan [a], Maria Elisa Calcagnotto [b], Beatriz Monteiro Longo [a,*]

[a] Laboratório de Neurofisiologia, Universidade Federal de São Paulo - UNIFESP, São Paulo, Brazil
[b] Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, Brazil

ARTICLE INFO

Article history:
Received 28 June 2016
Revised 7 March 2017
Accepted 7 March 2017
Available online 17 April 2017

Keywords:
Pentylenetetrazole
Maximal electroshock
Neurosphere
Medial ganglionic eminence
Acute seizure

ABSTRACT

Medial ganglionic eminence (MGE) is one of the sources of inhibitory interneurons during development. Following transplantation in postnatal developing brain, MGE cells can increase local inhibition suggesting a possible protection to GABAergic dysfunction in brain disorders, such as epilepsy. Since it has been shown that MGE-derived cells harvested as neurospheres are able to suppress seizures, it might be important to investigate whether these protective effects would change in different seizure models. Here, we used pentylenetetrazole- (PTZ) and maximal electroshock (MES)-induced seizure models to test whether the transplantation of MGE cells would increase the threshold to trigger acute seizures. When transplanted into the neocortex (layers 3–4) of neonatal mice (postnatal days 3–4), MGE cells were able to survive and were mainly found in piriform cortex, fimbria, and ventricular wall regions. Additionally, the number of GFP+ cells found in the brains of mice induced with PTZ and MES differed significantly and suggests proliferation and larger survival rate of MGE-transplanted cells after PTZ, but not MES-induced seizures. Following transplantation, there was a reduction in the number of animals presenting mild and severe seizures induced by PTZ. Furthermore, MGE-cell transplantation was able to increase threshold to seizures induced by PTZ, but was not able to prevent seizure spread induced by MES.

1. Introduction

During early stages of telencephalic development, cells from medial ganglionic eminences (MGE) tangentially migrate and originate cortical and hippocampal interneurons [1–4]. When transplanted into the brain, MGE-derived precursor cells preserve their ability to differentiate into inhibitory neurons and establish new synapses onto pre-existing neurons, integrating the neuronal network of the host brain [5–7]. In addition, these cells are effective in increasing the local inhibition promoting cortical plasticity in postnatal developing brain [8], suggesting a possible strategy of intervention in central nervous system (CNS) disorders related with GABAergic dysfunction, such as epilepsy [9–12], anxiety [13,14], and Alzheimer's disease [15]. MGE cells can be harvested in culture as cell aggregates known as neurospheres [16]. Neurospheres from MGE have shown anticonvulsive effects when transplanted into epileptic animals [11,17]. However, it is unknown whether this anticonvulsive effect of neurosphere-obtained MGE grafted cells would suppress different types of acute seizures *in vivo* after challenged by different convulsive stimuli.

Two experimental models of acute seizure induction have been widely used to verify the potential of antiepileptic drugs: the pentylenetetrazole (PTZ) and the maximal electroshock (MES) seizures models [18]. Pentylenetetrazole induces clonic or tonic–clonic seizures in rodents by acting on $GABA_A$ receptors and impairing the local inhibition mediated by GABA [19,20]. In the MES model, generalized tonic–clonic seizures are observed after applying an alternating current [18,21,22]. Since transplantation of neurosphere-obtained MGE cells is increasingly used in epilepsy studies due to its protective effect, we aimed to compare the *in vivo* effects of transplanted animals in two different models of acute seizure induction; the MES and PTZ models. Based on the idea that the animals transplanted with MGE cells would have a greater supply of GABAergic cells [5–8], we evaluated the possible protection of MGE grafts obtained from neurospheres in seizure susceptibility. We assessed whether these MGE transplanted cells produced distinct levels of protective effects modifying the threshold to trigger seizures that may influence the overall protection against seizures.

* Corresponding author.
E-mail address: biamonteironeuro@gmail.com (B. Monteiro Longo).
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.yebeh.2017.03.015
1525-5050/

2. Materials and methods

All animals were bred, raised, and maintained in the Center for the Development of Experimental Models in Medicine and Biology of the Universidade Federal de São Paulo. Mice were housed in polypropylene home cages (41 × 34 × 16.5 cm) in a pathogen-free facility. Adult male C57/Bl6 mice (weighing 30 to 35 g) were housed under controlled temperature (22–23 °C) and lighting (12 h light, 12 h dark, lights on at 6:45 a.m.) conditions. Rodent chow and water were available *ad libitum*. Experimental protocols were approved by the Animal Care and Ethics Committee of UNIFESP/SP (#1851/08).

2.1. MGE extraction and neurosphere culture preparation

Transgenic mice expressing enhanced green fluorescent protein (GFP) cDNA under the control of a chicken β-actin promoter and cytomegalovirus enhancer, provided by CEDEME (Center for the Development of Animal Models in Biology and Medicine at Universidade Federal de São Paulo), were used as MGE donors. Pregnant female mice were euthanized with a lethal dose of anesthetic (thiopental 50 mg/kg i.p., Cristalia, Brazil) ant the fetuses were taken from the uterus. Using a fluorescence microscope (Nikon), GFP+ fetuses were selected, and the EGM was dissected using curved forceps and a stereomicroscope (Nikon).

For tissue extraction and cell dissociation, ventricular and subventricular layers of MGE were dissected. Tissue was removed and incubated in trypsin (Gibco, USA) for 5 min at 37 °C followed by inactivation with fetal bovine serum (FBS, Gibco). After dissociation, cell number and viability were determined by trypan blue staining (Gibco) and reached a final viability of 90%. Cells were cultivated as neurospheres in a density of 100.000 cells/mL and the culture medium consisted of DMEM/F-12 (Gibco) supplemented with 1% N2 (100× Invitrogen, USA), 1% L-glutamine (200 mM, Invitrogen), 0.1% EGF (20 ng/mL, Sigma, USA), 0.05% FGF-2 (10 ng/mL, R&D Systems, USA) and 1% penicillin (100 IU/mL,), 1% streptomycin (100 μg/mL) and 1% amphotericin B (0.25 μg/mL) (all in antibiotic-antimycotic solution 100×, Invitrogen), 0.1% de gentamycin (Garamicina, Shering-Plough, Brazil). Neurospheres were maintained in culture for 5 to 8 days before transplantation.

2.2. Transplantation of cells dissociated from neurospheres

Neurospheres were incubated in trypsin for 5 min at 37 °C, following inactivation with FBS. Cells were dissociated, centrifuged, and resuspended in DMEM-F12. Suspension of dissociated neurosphere cells was placed in a Narishige microinjector guided by stereotaxic apparatus. Male C57/Bl6 pup mice (PN 3-4) were anesthetized by hypothermia (−4 °C) and 5–15 × 10^4 of GFP+ cells in 0.4 μL were bilaterally injected at a 45° angle into the neocortex (layers 3–4) of each mouse [14]. Sixteen neonatal mice (postnatal days 3–4) received MGE-derived cells (MGE groups), 18 age-matched control mice were injected at the same site and volume with culture medium (CTRL groups), and another group was injected with dead cells (by repeated frost and thaw cycles), as a control of inflammatory response (DC groups, n = 22). After cell transplantation, mice were returned to their mothers and kept in a thermal blanket at 37 °C. To avoid differences in basal stress, animals from control and transplanted experimental groups were obtained from the same litter, and all mice stayed away from their mothers for the same period.

2.3. Seizure induction

Seizures were induced by MES or PTZ in MGE, CTRL or DC transplanted mice at the age of 60 days. In both models, seizures were classified using the modified Racine scale and the analysis was performed considering seizures I–III as mild and IV–V as severe by a person who was blinded to the treatment and study hypothesis [23].

Briefly, mild seizures were identified as akinesia and piloerection (type I), facial jerking and neck and head jerks (type II), and clonic seizure of forelimbs (type III); and severe seizures were characterized by rearing or lying on their sides with forelimb and/or hindlimb clonus (type IV) and tonic and/or tonic–clonic hindlimb seizures (while lying on their sides) (type V) [23].

For the PTZ protocol, mice received 60 mg/kg s.c. injection of PTZ (Sigma) and were observed for 15 min. The dose was chosen based on the ability to induce tonic–clonic seizures in 80% of naïve mice tested initially for the standardization of the dose. The CTRL, DC, and MGE cell groups receiving PTZ s.c. injection were respectively named as CTRL-PTZ (n = 9), DC-PTZ (n = 11), and MGE-PTZ (n = 9). During 15 min of observation, latency to present seizure and the percentage of animals that had mild or severe seizures were evaluated. Mice that did not have any type of seizure were considered as 900 s, which correspond to the total observed period.

For the MES protocol, the CTRL, DC, and MGE cell groups subjected to MES induction were named as CTRL-MES (n = 9), DC-MES (n = 11), and MGE-MES (n = 7), respectively. An electrical stimulus (65 mA; 60 Hz; 0,15 s of duration) was applied through a pair of corneal electrodes (AVS Solução Integrada, Brazil) to induce acute generalized tonic–clonic seizure [21]. The duration of mild and severe seizures was analyzed for 15 min and the percentage of animals protected against seizure was calculated.

2.4. Immunohistochemistry

Animals were deeply anesthetized with Thiopental (50 mg/kg i.p., Cristalia, Brazil) and perfused through the heart with 100 mL of phosphate buffered saline (PBS) followed by 250 mL of 4% paraformaldehyde. To identify inhibitory neurons originated from the transplanted MGE neurosphere-derived GFP cells, sections were double-stained with a marker for a subtype of cortical interneurons parvalbumin (PV, 1:2000, Sigma) and GFP (anti-GFP AlexaFluor 488-conjugated antibody, 1:600; Molecular Probes/Invitrogen, USA. For the marker PV, we incubated the sections with rabbit IgG AlexaFluor 546-conjugated secondary antibodies (1:600, Molecular Probes) for 2 h. Sections were mounted using a nuclear-counterstaining fluorescence-preserving mounting medium containing DAPI (Vector, USA). Slides were examined using fluorescent microscopy (Nikon 80i), and images captured using the Nikon ACT-1 v.2 system. The GFP^+ cells were counted in 5 coronal sections per animal in which non-overlapping 10 fields were captured and quantified under 20× magnification.

2.5. Statistical analysis

The percentage of animals with mild or severe seizures induced by PTZ and MES were calculated and analyzed using Chi-Square test. Latency to mild or severe seizures after PTZ and the number of seizures after MES were evaluated using Kruskall-Wallis test. Statistical analysis of seizures and GFP cell counting was performed using GraphPad Prism 5. A significance level of 5% was assumed. Data are shown as mean ± SEM.

3. Results

3.1. MGE-neurosphere grafted cells increased seizure threshold induced by PTZ but had no effect in preventing seizure spread induced by MES

Mild seizures were observed in 89% of CTRL-PTZ group and in 82% of DC-PTZ group. In contrast, only 33% of the animals from MGE-PTZ group had mild seizures (Chi-Square, p = 0.0197, Fig. 1A). Likewise, severe seizures were observed in 78% of CTRL-PTZ group and in 73% of DC-PTZ group, while only 22% of MGE-PTZ had severe seizures (Chi-Square,