Journal of Neuroscience Methods 201 (2011) 307–314

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

A comparative analysis of intraperitoneal versus intracerebroventricular administration of bromodeoxyuridine for the study of cell proliferation in the adult rat brain

M. Cifuentes[a], M. Pérez-Martín[a], J.M. Grondona[a], M.D. López-Ávalos[a], N. Inagaki[c], P. Granados-Durán[a], P. Rivera[b], P. Fernández-Llebrez[a,*]

[a] Departamento de Biología Celular, Genética y Fisiología, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain
[b] Laboratorio de Medicina Regenerativa, Hospital Carlos Haya, Fundación IMABIS, Avda. Carlos Haya 82, Pabellón de Gobierno, 29010 Málaga, Spain
[c] Department of Diabetes and Clinical Nutrition, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

ARTICLE INFO

Article history:
Received 31 January 2011
Received in revised form 5 August 2011
Accepted 7 August 2011

Keywords:
Adult neurogenesis
Circumventricular organs
Brain capillaries
Cell proliferation

ABSTRACT

Bromodeoxyuridine (BrdU) is the most widely used marker to detect proliferative cells in the adult brain. Here we analyse whether the route of administration of the tracer influences the number of labelled cells. For the intraperitoneal (ip) administration of BrdU, we performed two daily injections during 7 days, and for an intracerebroventricular (icv) delivery, it was continuously infused into one lateral ventricle for a 7 days period as well. After ip administration, cells labelled with BrdU were seen in the subventricular zone of the striatal wall of the lateral ventricle, the hippocampus and the neurohemal circumventricular organs. Also, the habenula and large myelinated tracts, such as the fornix and the corpus callosum, showed many BrdU-positive nuclei. Labelled nuclei were scarce in the parenchymal regions of the rest of the brain. In contrast, a significant increase in the number of BrdU-positive nuclei was observed in the parenchyma of the periventricular zones after icv administration of the marker, thus showing a greater availability of the tracer when it was administered directly into the ventricular cerebrospinal fluid. We suggest that the availability of BrdU in the vicinity of proliferating cells may depend on the permeability of the brain vessels to nucleosides in each location. By using double immunocytochemistry we found that neurons, astrocytes, oligodendrocytes, tanycytes and microglia had incorporated the tracer, demonstrating their proliferation capacity.

1. Introduction

5-Bromo-2′-deoxyuridine (BrdU) is the most widely used marker to study cell proliferation in the adult brain (Kempermann, 2006). It is incorporated into the newly synthesized DNA during the S phase in the place of deoxythymidine, and can be easily detected using specific antibodies. After administration of BrdU, it is assumed that the number of labelled nuclei reflects the intensity of cell proliferation at a given place. However, the availability of BrdU greatly depends on the administration protocol, that is, the route of delivery, the scheduled timing, the permeability of the barriers found between the compartment of delivery and the location of the cells under study, and the potential clearance capacity of the tissue for BrdU (Spector and Johanson, 2007).

In most studies on cell proliferation in the adult brain, BrdU was administered peripherally by intraperitoneal (ip) or intravascular injection, or even in the drinking water. In these cases it was assumed that BrdU enters the brain through the blood–brain barrier (BBB) or the blood–cerebrospinal fluid (CSF) barrier (i.e. the ependymal cells of the choroid plexus). Equilibrative nucleoside transporters located in the endothelial cells of the brain vessels and in the ependymal cells of the choroid plexuses, make possible the transport of BrdU from the blood to the intercellular spaces of the brain parenchyma by facilitated diffusion (Redzic et al., 2005; Spector and Johanson, 2007). On the other hand, concentrative nucleoside transporters, located in the abluminal membrane of the endothelial cells of the brain vessels and in the apical membrane of the ependymal cells of the choroid plexuses, would be responsible for the rapid clearance of nucleosides from the intercellular spaces of the brain and from the ventricular CSF by active transport (Redzic et al., 2005; Spector and Johanson, 2007). However, it is not known

Abbreviations: AP, area postrema; BBB, blood–brain barrier; BrdU, 5-bromo-2′-deoxyuridine; CNS, central nervous system; CSF, cerebrospinal fluid; CVO, circumventricular organ; GFAP, glial fibrillary acidic protein; ip, intraperitoneal; icv, intracerebroventricular; ME, median eminence; OVLT, organon vasculosum of the lamina terminalis; SCO, subcommissural organ; SFO, subfornical organ; SGZ, subgranular zone; SVZ, subventricular zone.

* Corresponding author. Tel.: +34 9 52131858; fax: +34 9 52132000.
E-mail address: llebrez@uma.es (P. Fernández-Llebrez).

0165-0270/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2011.08.006

whether these nucleoside transport systems operate similarly in different brain zones and, consequently, if the BrdU administered in the periphery is equally available in all regions of the adult brain.

Neurogenesis in the central nervous system (CNS) of adult rodents is prominent in two main locations: the subventricular zone (SVZ) of the striatal wall of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus (Ming and Song, 2005). More recently it was shown that the circumventricular organs contain neural stem cells that give rise to neurons and glia (Bennett et al., 2009, 2010). To a lower extent, cell proliferation was reported as well in many other periventricular regions of the brain and the spinal cord of adult rodents, sometimes after the stimulation with growth factors (Pencea et al., 2001; Chouaf-Lakhdar et al., 2003; Xu et al., 2005; Danilov et al., 2006; Kokoeva et al., 2005, 2007; Pérez-Martín et al., 2010). In an interesting study, Kokoeva et al. (2007) showed that the number of BrdU-labelled cells in the hypothalamus of mice dramatically increased when BrdU was administered through a cannula inserted into one lateral ventricle compared to animals that were injected ip.

The aim of the present investigation was to study cell proliferation in different regions of the brain of the adult rat after BrdU administration either ip or intracerebroventricularly (icv). We compared the proliferation observed in the highly permeable neurohemal regions with that in other zones with a well-developed BBB. We also evaluated the nature of the newborn cells, and showed that neurons, astrocytes, tanycytes, oligodendrocytes and microglia are constitutively generated in several regions of the adult rat brain.

2. Materials and methods

2.1. Animals

Adult male Wistar rats ($n = 10$) weighting about 200 g (Charles River Laboratories, Wilmington, MA) were used in this study. Animals were kept under a photoperiod of 12L:12D with free access to food and water. Manipulation of the animals followed the principles of laboratory animal care published by Spanish Ethical Committee (RD 1201/2005) and the European Union normative (86/609/EEC).

2.2. Implantation of the intracerebroventricular cannula

The rats were anaesthetized with 2,2,2-tribromoethanol (300 mg/kg body weight). An osmotic minipump (flow rate 1 μl/h; Alzet 2001, Alza, Palo Alto, CA, USA) was subcutaneously implanted between the scapulae, and maintained for 7 days. The minipump was attached to a brain infusion cannula (Alza) through polyethylene tubing and primed by immersion in normal saline at 40 °C for 4 h. These procedures were conducted under sterile conditions. The cannula was implanted into the right lateral cerebral ventricle (−0.5 mm anteroposterior, −1.4 mm lateral and −3.3 mm dorsoventral; coordinates based on Paxinos and Watson, 1986) and secured to the skull with dental cement. The minipumps was filled with saline or BrdU solution.

2.3. Experimental groups

2.3.1. Group 1 (G1): intracerebroventricular saline plus intraperitoneal BrdU

Four rats received saline (0.9% NaCl, 1 μl/min) for 7 days into the right lateral ventricle while BrdU (Sigma) was ip injected (10 mg/ml in 0.9% NaCl, 50 mg/kg body weight) twice per day (every 12 h, early in the morning and late in the evening) during the same 7 days. This represents a total amount of 140 mg per animal during the 7 days. Animals were sacrificed 12 h after the last BrdU injection.

2.3.2. Group 2 (G2): intracerebroventricular BrdU

Four rats were icv administered with BrdU (1 mg/ml in 0.9% NaCl) continuously infused for 7 days through a cannula as described above (flow rate 1 μl/h). This represents a total amount of 0.17 mg per animal during the 7 days.

2.3.3. Group 3 (G3): intraperitoneal BrdU

Two rats were ip injected with BrdU, as described above for experimental group 1, but without icv cannula implantation.

2.4. Tissue processing and immunohistochemistry

The animals were anaesthetized with 2,2,2-tribromoethanol and perfused through the aorta with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB) pH 7.4. Brains were removed from the skull, immersed in the same fixative for 4 h at 4 °C, and rinsed with PB. Coronal sections (50 μm thick) were obtained with a vibratome (Leica Heidelberg, Germany).

2.4.1. BrdU immunohistochemistry

For BrdU detection we used a monoclonal mouse anti-BrdU antibody. This is an IgG_1 monoclonal antibody obtained from ascites by using BrdU-bovine serum albumin (72–73 kDa) as immunogen. Dilution: 1:5000. Source: Hybridoma Bank, Iowa City, IA, USA; Ref. G3G4. This antibody was previously used for studies on rat CNS cell proliferation (Pérez-Martín et al., 2010; Rivera et al., 2011; for more information about the antibody and references about the use of this antibody go to http://dshb.biology.uiowa.edu/G3G4AntiBrdUrd).

For immunostaining, the endogenous peroxidase activity in the tissue sections was first quenched by incubation in 3% H_2O_2 and 10% methanol in PB for 30 min at room temperature (RT). As a previous antigen retrieval step, DNA was denatured by incubating the sections in 2 N HCl for 15 min at 37 °C, and rinsed twice in 0.1 M borate buffer (pH 8.5). Blocking of non-specific binding sites was done by an incubation step with PST (0.3% Triton X-100, 0.3% bovine serum albumin in 0.1 M PB). 0.1 M PB buffer was used in all the following washes and PST for the dilution of the antibodies. Sections were incubated overnight at 4 °C in agitation in primary antibody. After rinsing, sections were incubated for 90 min with biotinylated goat anti-mouse IgG (1:1000, Pierce, Rockford, IL, USA), rinsed and transferred to the avidin–biotin peroxidase complex solution (1:250; Pierce) for 45 min. Peroxidase was detected using a solution of 0.05% diaminobenzidine (DAB, Sigma) as chromogen. In control sections incubation with the primary antibody was omitted.

2.4.2. Double immunofluorescence

Sections obtained from animals of group 2 were used for the identification of the proliferationg cells using the following antibodies: rat anti-BrdU (1:2000; Accurate Scientific, Westbury, NY, USA), mouse anti-GFAP (1:2000; Sigma) for the detection of astrocytes, mouse anti-vimentin (1:2000; Sigma) for ependymocytes, mouse anti-NeuN (1:1000, Chemicon, Temecula, CA) for neurons, rabbit polyclonal anti-Iba-1 (1:1000, Wako Chemical USA Inc., Richmond, VA, USA) for microglia; rabbit anti rat-ABCA2 (Zhou et al., 2001) for oligodendrocytes. For the detection of BrdU-labelled nuclei, DNA was denatured as mentioned above, and then the sections were incubated overnight at 4 °C with a combination of the selected primary antibodies. After washing in PB, sections were incubated for 2 h with the secondary antibodies diluted 1:1000: goat anti-rat IgG labelled with Alexa 488, donkey anti-mouse IgG Alexa 594, or donkey anti-rabbit IgG Alexa 594 (Molecular Probes). The labelled sections were visualized with a confocal microscope (Leica Microsystems). In some sections nuclei were stained with