Pharmacological Reports 67 (2015) 1208–1214

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Effects of neuroleptics administration on adult neurogenesis in the rat hypothalamus

Ewa Rojczyk *, Artur Pałasz, Ryszard Wiaderkiewicz

Department of Histology, Faculty of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

ARTICLE INFO

Article history:
Received 11 February 2015
Received in revised form 18 April 2015
Accepted 22 April 2015
Available online 2 May 2015

Keywords:
Neurogenesis
Olanzapine
Chlorpromazine
Haloperidol
Hypothalamus

ABSTRACT

Background: Among many factors influencing adult neurogenesis, pharmacological modulation has been broadly studied. It is proven that neuroleptics positively affect new neuron formation in canonical neurogenic sites – subgranular zone of the hippocampal dentate gyrus and subventricular zone of the lateral ventricles.

Latest findings suggest that adult neurogenesis also occurs in several additional regions like the hypothalamus, amygdala, neocortex and striatum. As the hypothalamus is considered an important target of neuroleptics, a hypothesis can be made that these substances are able to modulate local neural proliferation.

Methods: Experiments were performed on adult male rats injected for 28 days or 1 day by three neuroleptics: olanzapine, chlorpromazine and haloperidol. Immunohistochemistry was used to determine expression of proliferation marker (Ki-67) and the marker of neuroblasts – doublecortin (DCX) – which may inform about drug influence on adult neurogenesis at the level of the hypothalamus.

Results: It was shown that a single injection of antipsychotics causes significant decrease in hypothalamic DCX expression, but after chronic treatment with chlorpromazine, but not olanzapine, there is an increase in the number of newly formed neuroblasts. Haloperidol has the opposite effect – its long-term administration decreases the number of DCX-positive cells. Cell proliferation levels (Ki-67 expression) increase after long-term drug administration, whereas their single doses do not have any modulatory effect on proliferation potential.

Conclusions: Our results throw a new light on the neuroleptics mechanism of action. They also support the potential role of antipsychotics as a factor that can modulate hypothalamic neurogenesis with putative clinical applications.

Introduction

Adult neurogenesis appears to occur in specific brain regions, being considered a unique phenomenon in the processes of memory, learning and neural plasticity. In the adult mammalian brain, neural stem cells are located mainly in the subventricular zone (SVZ) of the lateral ventricle ependymal wall and in the subgranular zone (SGZ) of the hippocampal dentate gyrus. At present, accumulating data proves that new neural cells can also be formed, usually in limited number, outside the canonical sites, in potential niches located in distinct structures of a mature brain. Their presence has been proven in the hypothalamus [1–3], amygdala [4], substantia nigra [5], striatum [6] and even neocortex [7]. However, the presence of established and permanently functional neural stem cells (NSCs) niches in the aforementioned brain areas is still quite controversial. A hypothesis suggesting existence of a stable hypothalamic neurogenic site located in the subependymal zone of the third ventricle (hypothalamic ventricular zone, HVZ) seems to be especially intriguing and relatively well-documented [1,8]. More importantly, it is also suggested that hypothalamic neurogenesis is significantly involved in various regulatory mechanisms, particularly in energy balance regulation [8,9].

A large spectrum of endogenous regulatory substances, especially growth factors, are able to regulate the course of adult hypothalamic neurogenesis. The brain-derived growth factor (BDNF), insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), fibroblast growth factor (FGF) and ciliary neurotrophic factor (CNTF) seem to play a key role as the activators of neuronal progenitor divisions in different brain regions [10–13].

* Corresponding author.
E-mail address: ewarojczyk@gmail.com (E. Rojczyk).

http://dx.doi.org/10.1016/j.pharep.2015.04.014
1734-1140/

A recent report suggests, that also gonadotropin-releasing hormone (GnRH) can increase the proliferating activity of hypothalamic neural progenitor cells (NPCs) in aged mice [14]. It should be emphasized that aforementioned growth factors promote neurogenesis in different hypothalamic centres in a distinctly selective manner [15]. Thus, BDNF administration induces neurogenesis in the paraventricular nucleus, both FGF and CNTF increase cell proliferation in the parenchymal zone of the arcuate nucleus, while IGF-1 in the medial periventricular region [9,16]. Interestingly, extended subcutaneous injection of FGF and EGF increases neurogenesis in the arcuate nucleus, whereas a single simultaneous intracerebroventricular injection of these factors promotes cell proliferation in the ependymal layer [2].

Even more data suggest the possibility to modulate neurogenesis in classical neurogenic niches (SVZ and SGZ), both by endogenous and exogenous substances. The origin of neurons in these regions can be precisely regulated not only by various growth factors [10,12,13,17], neurotransmitters [18], cytoskeleton proteins [19] and neuropeptides [20,21], but also by commonly administered pharmaceuticals such as selective serotonin reuptake inhibitors [22,23], normothymic drugs [24], antipsychotics [25–29], lithium ions [30] and even steroid hormones [31]. Moreover, potential connections between adult neurogenesis and pathogenesis and/or the course of bipolar disorder [32], schizophrenia [33,34], and particularly depression [35,36] are also broadly discussed.

It is proven that chronic treatment with antipsychotics (neuroleptics) promotes new neural cells survival and increases the number of NSCs in different brain regions, especially in classical neurogenic sites. This effect is most evident after administration of atypical antipsychotics like olanzapine (OLZ) and clozapine [29,37–41], whereas data about a typical antipsychotic – haloperidol (HAL) – are not so consistent and suggest different effects dependent on the experimental model [25–28,38,42].

Nevertheless, there is no information concerning the influence of neuropsychiatric drugs (including antipsychotics) on the course of hypothalamic neurogenesis. If we consider that the hypothalamus may be an important target for most antipsychotics, than a modulatory effect of these substances on neurogenesis in SVZ/SGZ makes us hypothesize that intraperitoneal injection of some neuroleptics could affect adult rat hypothalamic neurogenesis. Thus, we performed experiments using a rat model. The goal was to determine the effect that chronic and single-dose administration of three types of neuroleptics – OLZ, chlorpromazine (CPZ) and HAL – would have on the origin of new neural cells by counting doublecortin (DCX)-positive neuroblasts (DCX^+) and mitotically active nuclear antigen Ki-67-expressing cells ($Ki\text{-}67^+$). DCX detects immature neurons and is broadly used as a specific marker reflecting the level of adult neurogenesis [1,43,44], whereas Ki-67 is expressed during all active phases of a cell cycle, so it is used to detect proliferating cells in tumours, but also during adult neurogenesis [9,45,46].

Materials and methods

Animals

The studies were performed on adult (2–3 months old, 180–210 g) male Sprague-Dawley rats from the Medical University of Silesia Experimental Centre, housed at 22 °C with a regular 12/12 light–darkness cycle with access to standard Murigan chow and water *ad libitum*. We specified 8 experimental groups (each comprised of four rats), which gave 32 animals for the whole experiment. All experimental procedures were approved by the local bioethic committee at the Medical University of Silesia (agreement 36/2012).

Neuroleptics administration and material collection

Four groups of animals (C, OLZ, CPZ, HAL) received a control vehicle – saline (C) and respectively OLZ 5 mg/kg/day, CPZ 5 mg/kg/day and HAL 2 mg/kg/day by intraperitoneal injections every day for 4 weeks (28 injections). A further four groups (Cs, OLZs, CPZs, HALs) were treated with antipsychotics in the same pattern, but only with a single injection. The above-mentioned non-toxic doses of drugs were established on the basis of pharmacological standards developed in preclinical studies. 24 h after the last drug administration (for the long-term study) and 24 h after single drug dose administration (for the short-term study), the animals were quickly anaesthetized with isoflurane inhalation and sacrificed. Then, the brains were excised, postfixed (with 4% paraformaldehyde in PBS, pH 7.2–7.4), dehydrated, embedded in paraffin and finally sectioned on the microtome (Leica Microsystems) in the coronal plane (−2.00 to −2.80 mm from bregma) at 7 μm slice thickness, according to Paxinos & Watson's The Rat Brain in Stereotaxic Coordinates (2007).

Immunohistochemistry and immunofluorescence

After rehydratation, antigen demaskation (in low pH antigen unmasking solution, Vector Laboratories, Burlingame, CA, USA) and blockage with 10% serum, brain sections (on slides) for each experimental group were incubated overnight in 4 °C with the goat antibody against DCX – marker of neuroblasts (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA) and with the rabbit antibody against Ki-67 – proliferation marker (1:100, Abcam, Cambridge, MA, USA). Each immunostained slide contained also a negative control – another (second) brain section incubated at the same time with goat/rabbit IgG instead of a primary antibody in order to check specificity of antibodies used. Incubation with primary antibodies was followed by administration of biotynylated secondary antibodies (1:200), and then an avidin–biotin–horseradish peroxidase complex (Vectastain ABC kit, Vector Laboratories). Finally, 3,3′-diaminobenzidine (DAB) was used (peroxidase substrate kit DAB, Vector Laboratories) to complete the reaction and to visualize neuroblasts (DCX^+) and newly formed cells ($Ki\text{-}67^+$). For calculation of DCX-positive cells, 20 slices (every fifth one) per rat were used. We did the same for Ki-67. All images (8 per slice) were captured and analyzed with Nikon Coolpix optic systems. Immunopositive cells were counted from the whole hypothalamic area of each slice using ImageJ 1.43u software.

Additionally, after overnight incubation with primary antibodies for DCX and Ki-67, several brain sections were kept in darkness with secondary antibodies labelled with fluorochromes – TRITC and FITC respectively (1:200, anti-goat with TRITC, anti-rabbit with FITC, Abcam). Then, a mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) was used in order to label cell nuclei and obtain two-colour staining of DCX^+ and $Ki\text{-}67^+$ cells.

Statistical analysis

Statistical analyses were performed using Statistica (Systat Software, San Jose, CA, USA). Data are presented as mean ± SEM. Mean differences between groups were analyzed using nonparametric Kruskal–Wallis test. Differences were considered statistically significant at $p < 0.05$.

Results

Negative control analysis revealed that all antibodies used were specific and selective – omission of the primary antiserum resulted in complete loss of the immunostaining.

DCX and Ki67-positive cells were widely dispersed throughout the hypothalamus. However, the local patterns of expression of these markers were clearly visible. DCX (Figs. 2 and 3) was mainly