Neuroscience Letters 506 (2012) 39–43

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Unchanged expression of the ceramide transfer protein in the acute 6-OHDA neurodegenerative model

Chiara Mencarelli[a,d], Gerard H. Bode[a,d], Rinske Vlamings[a,d], Marcus L.F. Janssen[a,d], Mario Losen[a,d], Marc H. De Baets[a,b,d], Harry W.M. Steinbusch[a,d], Yasin Temel[a,c,d], Pilar Martinez-Martinez[a,d,*]

[a] Department of Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands
[b] Neuroimmunology Group, Biomedical Research Institute (BIOMED), Hasselt University, Diepenbeek, Belgium
[c] Department of Neurosurgery, University Hospital Maastricht, Maastricht, The Netherlands
[d] European Graduate School of Neuroscience (EURON), The Netherlands

ARTICLE INFO

Article history:
Received 10 June 2011
Received in revised form
21 September 2011
Accepted 15 October 2011

Keywords:
Parkinsons's disease (PD)
Ceramide transporter (CERT)
Goodpasture-antigen binding protein (GPBP)
6-Hydroxydopamine (6-OHDA)
Ceramide
Neurostereology

ABSTRACT

Ceramides are lipids that are abundant in brain tissue where they have an important structural role in cellular membranes. Ceramides are also powerful intracellular signalling molecules controlling cell death, growth and differentiation. So far, the ceramide transfer protein (CERT), a shorter splice variant of the Goodpasture antigen-binding protein (GPBP), is the only known protein with the ability to shuttle ceramide from the endoplasmic reticulum to the Golgi apparatus. GPBP/CERT are widely distributed in the central nervous system where they act as key factors for normal brain development and homeostasis. Ceramide accumulates in neurons during acute neurodegeneration. The objective of this study was to define whether levels of the ceramide transfer protein GPBP/CERT are altered in the acute neurodegenerative process. We used design-based stereology to quantify the number of GPBP/CERT immunoreactive cells in the striatum of 6-hydroxydopamine (6-OHDA) lesioned rats as an animal model of Parkinson's disease (PD). In addition, gray value measurement was performed to quantify GPBP/CERT immunoreactivity-levels within individual cells. No difference in the striatal expression levels of GPBP/CERT proteins was found between diseased and control animals, suggesting that the expression pattern of GPBP/CERT in the striatum is not affected in the 6-OHDA rat model of PD.

1. Introduction

Ceramides are lipids abundant in brain tissue where they were first discovered by Johann L.W. Thudichum in 1884 (for a review [38]). They have an important structural role in cellular membranes and also act as signalling molecules inside the cell [1]. The balance of ceramide levels extensively regulates cell functions [14].

Due to their hydrophobic nature, ceramides were thought to function exclusively at the site of their synthesis. The existence of a protein capable of transferring ceramide between two different locations in the cytoplasm was demonstrated in 2003 by Hanada and colleagues [17]. The ceramide transfer protein (CERT), the shorter splice variant of the Goodpasture antigen-binding protein (GPBP) [28] shuttles ceramide between the endoplasmic reticulum (ER) and the Golgi in a non-vesicular manner. The ceramide transporter GPBP/CERT is widely distributed in the brain with higher expression levels in neurons compared to other cell types [25].

Ceramide levels are increased in neurodegenerative diseases such as Alzheimer's disease [10], Parkinson's disease (PD) [9], dementia with Lewy bodies and amyotrophic lateral sclerosis [11]. Ceramides are augmented in the cerebrospinal fluid of Alzheimer's disease patients and serum ceramides are early predictors of cognitive impairment in Alzheimer's disease [30].

We hypothesised that the protein levels of the ceramide transporter also change during acute neurodegeneration.

To study GPBP/CERT levels in a neurodegenerative condition, we used an experimental model to mimic the dopamine depletion. Rats were chronically dopamine (DA) depleted by bilateral striatal injections of 6-hydroxydopamine (6-OHDA). 6-OHDA, being similar to DA, shows high affinity for the dopamine transporter, which carries this neurotoxin inside the dopaminergic neurons. 6-OHDA accumulates in the cytosol and undergoes prompt auto-oxidation, promoting a high rate of free radical formation [12]. As a consequence intra-striatal 6-OHDA injection permanently damages the dopaminergic nigrostriatal pathway. The loss of dopaminergic input to the striatum disrupts the basal ganglia circuit and is responsible for the most prominent symptoms of PD in the extent

* Corresponding author at: Department of Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands. Tel.: +31 43 3881042; fax: +31 43 3671096.
E-mail address: p.martinez@maastrichtuniversity.nl (P. Martinez-Martinez).

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.10.044

that the degree of neurological deficit is related to the loss of striatal DA [18].

Nigral dopaminergic neurons are naturally exposed to oxidative stress because in the presence of molecular oxygen DA undergoes spontaneous oxidation, leading to the formation of reactive oxygen species (ROS) [15,23]. In turn, conditions known to promote elevated cellular levels of ROS can lead to ceramide accumulation [19] which is associated with the induction of apoptotic cell death [6,13,38].

Here, we have conducted a design-based stereology investigation to quantify GPBP/CERT immunoreactive cells in the striatum of rats injected with 6-OHDA.

2. Material and methods

All experimental procedures were approved by the Animal Experiments and Ethics Committee of Maastricht University. Animal and surgical procedures used have been described in detail before [37]. Fourteen male Lewis rats at the age of 12 weeks received stereotactic bilateral injections at two sites per hemisphere with either 2 microl of 6-OHDA (5 μg/μl dissolved in 0.9% saline and 0.2% ascorbic acid; Sigma, Zwijndrecht, The Netherlands) or saline (0.9% saline and 0.2% ascorbic acid) in the striatum. To protect noradrenergic neurons from 6-OHDA, the rats received 20 mg/kg desipramine 1 h before surgery.

After 2 weeks post injection, rats were perfused transcardially. Brains were removed, postfixed, frozen and cut serially in 30-μm thick coronal sections [25].

Immunohistochemistry for GPBP/CERT was carried out as described previously [25]. For the stereologic procedures, a stereology workstation consisting of an Olympus BX51 microscope (Olympus, Tokyo, Japan), motorized specimen stage, color video camera and a PC with StereoInvestigator stereology software (MBF Bioscience, Vermont, USA) was used.

The number of cells expressing GPBP/CERT and labelled with the abovementioned polyclonal antibody were quantified in a part of the striatum, with a rostral boundary at Bregma 1.60 mm where the corpus callosum crosses the midline and a caudal boundary at Bregma −0.90 mm where the fornix enters the diencephalon. The dorsal and lateral boundaries of this region consisted of the corpus callosum and the medial boundary was defined by the lateral ventricle. A ventral boundary was set by drawing a line from the tip of the lateral ventricle to the rhinal fissure [20]. The region of interest was delineated using a 4× objective.

The Optical Fractionator technique [33] was used to determine the total numbers of cells positive for GPBP/CERT. Positive cells were counted using a 100× objective in unbiased virtual counting spaces distributed in a systematic random fashion throughout the delineated region of interest. The total number of positive cells in the region of interest was estimated as a function of the number of cells counted and the sampling probability [33].

For, quantifying the number of TH immunoreactive (THir) neurons, a similar stereological approach was used. After exactly tracing the boundaries of the substantia nigra pars compacta (SNc) on microscopic video images displayed on a monitor, numbers of neurons were evaluated with the Optical Fractionator [31]. Estimated total numbers of neurons were calculated from the numbers of counted neurons and the corresponding sampling probability. For more details see [37].

GPBP/CERT immunostained neurons were evaluated also by gray value. Mean gray values for regions of interest (Bregma 0.7 and −0.4) were calculated using ImageJ software, with images photographed at a magnification of 40×. The mean gray value of cells in the selected regions was used as a measurement for the expression levels of GPBP/CERT in the striatum.

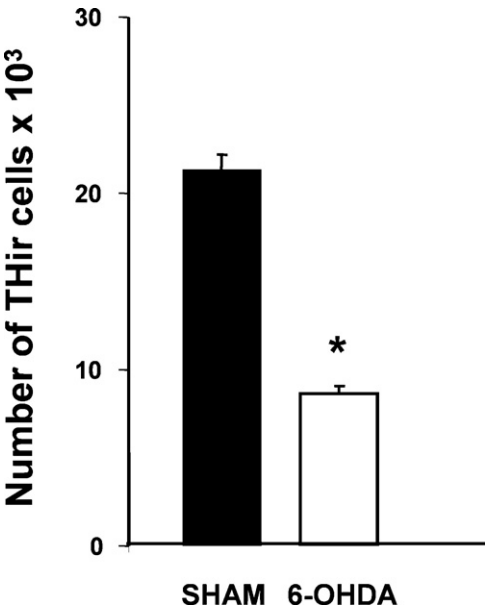

Fig. 1. Mean total numbers of THir-neurons of sham-operated rats and rats subjected to dopamine depletion. Data represent means and SEM per group. Since the left and right SNc showed a similar cell count, the data were pooled. The p values from the corresponding post hoc LSD tests are provided as $^{*}p < 0.05$.

The statistical analysis was done with GraphPad Prism (Version 5.01 for Windows, GraphPad Software, San Diego, CA). The mean and the standard error of the mean (SEM) were determined for the numbers of cells expressing GPBP/CERT and the striatal volume in both 6-OHDA and control rats. An unpaired two-tailed t-test ($p \leq 0.05$) was performed to compare the means of the 6-OHDA group and the control group (Table 1).

Three unilateral 6-OHDA lesioned rats were used (disease induction and handling similar to animals describes above). Brains were removed and striatal samples were dissected in two hemispheres the 6-OHDA-injected and from the control hemisphere, frozen and stored at −80 °C. The samples were quickly frozen with solid CO_2 and stored at −80 °C striatal brain homogenization is performed as described previously [25]. Proteins were separated by SDS-PAGE followed by electroblotting to nitrocellulose membrane (Millipore, Amsterdam Zuid-Oost, The Netherlands). Membranes were incubated with rabbit polyclonal anti-GPBP/CERT antibody (epitope 1–50 of human GPBP, Bethyl Laboratories, USA) and mouse monoclonal anti-GAPDH antibody (Ab9484, Abcam, Cambridge, UK). Subsequently the membrane was incubated with goat anti-rabbit-Alexa 800 and donkey anti-mouse-Alexa 680 (Rockland, USA). The mean intensity of the GPBP/CERT bands was measured in ImageJ and corrected with the mean intensity of the GAPDH band.

3. Results

Injections of 6-OHDA into the striatum resulted in a substantial loss of THir cells in the SNc as compared to the sham group. High-precision design-based stereological analysis revealed a significant 6-OHDA-induced reduction in the total number of THir cells in the SNc of about 65% bilaterally ($p < 0.01$) (Fig. 1).

6-OHDA affects first the striatum with dopamine nerve terminal disruptions and consequent progressive retrograde degeneration of nigro-striatal dopamine neurons in the SNc. Therefore in this study we selected the striatum as area of investigation (Fig. 2C), instead of the SNc where the massive death of dopaminergic neurons (Fig. 2A and B) would have masked the quantification of GPBP/CERT levels.