Neuroscience 256 (2014) 10–22

MODELING NIGROSTRIATAL DEGENERATION IN ORGANOTYPIC CULTURES, A NEW EX VIVO MODEL OF PARKINSON'S DISEASE

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Abstract—Parkinson's disease (PD) is the second most frequent neurodegenerative disorder afflicting 2% of the population older than 65 years worldwide. Recently, brain organotypic slices have been used to model neurodegenerative disorders, including PD. They conserve brain three-dimensional architecture, synaptic connectivity and its microenvironment. This model has allowed researchers a simple and rapid method to observe cellular interactions and mechanisms. In the present study, we developed an organotypic PD model from rat brains that includes all the areas involved in the nigrostriatal pathway in a single slice preparation, without using neurotoxins to induce the dopaminergic lesion. The mechanical transection of the nigrostriatal pathway obtained during slice preparation induced PD-like histopathology. Progressive nigrostriatal degeneration was monitored combining innovative approaches, such as diffusion tensor magnetic resonance imaging (DT-RMI) to follow fiber degeneration and mass spectrometry to quantify striatal dopamine content, together with bright-field and fluorescence microscopy imaging. A substantia nigra dopaminergic cell number decrease was observed by immunohistochemistry against rat tyrosine hydroxylase (TH) reaching 80% after 2 days in culture associated with a 30% decrease of striatal TH-positive fiber density, a 15% loss of striatal dopamine content quantified by mass spectrometry and a 70% reduction of nigrostriatal fiber fractional anisotropy quantified by DT-RMI. In addition, a significant decline of medium spiny neuron density was observed from days 7 to 16. These sagittal organotypic slices could be used to study the early stage of PD, namely dopaminergic degeneration, and the late stage of the pathology with dopaminergic and GABAergic neuron loss. This novel model might improve the understanding of PD and may represent a promising tool to refine the evaluation of new therapeutic approaches.

Key words: Parkinson's disease, nigrostriatal organotypic slices, MRI, diffusion tensor imaging, dopamine, mass spectrometry.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting 2% of the population older than 65 years, with 7–10 million people concerned worldwide (Lees et al., 2009; Gazewood et al., 2013). Clinically, the cardinal symptoms are tremor, rigidity of muscles, bradykinesia and loss of postural reflexes (Parkinson, 1817). The major symptoms are due, in part, to the lack of dopamine released in the striatum as a result of degeneration of dopaminergic neurons in the substantia nigra (SN). Dopaminergic neuronal death seems to be induced by α-synuclein aggregation, forming Lewy bodies which are toxic (Spillantini et al., 1997; Volles and Lansbury, 2003). At present, there is no cure for PD and treatments are merely symptomatic. Current therapy based on a dopamine replacement strategy consists mainly on the oral administration of the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA), but in long-term administration some secondary effects may appear (Ecker et al., 2009; Hauser, 2009). Novel drug and cell therapy approaches require extensive evaluation before routinely being used in humans (Poewe et al., 2012; Lindvall, 2013).

A wide variety of *in vitro* (Alberio et al., 2012) and *in vivo* models (Jackson-Lewis et al., 2012) have been developed to elucidate the pathogenesis, cell death mechanisms and to evaluate therapeutic strategies for PD. These PD models require a reproducible and well-characterized degeneration of the nigrostriatal dopaminergic system which is the main, but not the only pathway involved in PD. *In vivo* models can be broadly divided into genetic (Dawson et al., 2010) and neurotoxic models (Tieu,

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Abbreviations: 6-OHDA, 6-hydroxydopamine; BSA, bovine serum albumin; DARPP32, dopamine- and cAMP-regulated neuronal phosphoprotein; DT-RMI, diffusion tensor magnetic resonance imaging; FA, fractional anisotropy; H_2O_2, hydrogen peroxide; Hank's, Hank's Balanced Salt Solution; IgG, immunoglobulin G; LC–MS/MS, liquid chromatography–mass spectrometry; MEM, Minimum Essential Medium Eagle; MFB, medial forebrain bundle; MRI, magnetic resonance imaging; MSN, medium spiny neuron; NeuN, neuronal nuclei; NGS, normal goat serum; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline containing 1% Triton; PD, Parkinson's disease; RARE, rapid acquisition with relaxation enhancement; ROS, reactive oxygen species; SN, substantia nigra; TH, tyrosine hydroxylase.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.10.021

2011). However, *in vivo* studies require high technical and financial resources and they do not allow to simultaneously test several conditions in the same animal. Furthermore, it results in an important difficulty to address different engraftment mechanisms when testing cell therapy approaches. In contrast, *in vitro* experiments with dopaminergic neurons are easy to develop and use but they do not allow the study of cell interactions with the microenvironment or with the host tissue.

Recently, organotypic brain slice cultures have been used for PD modeling. Since their introduction, organotypic brain cultures have become a useful tool to study physiological properties of tissues, to monitor both acute and chronic effects of drugs and to study neurological disorders as in global cerebral ischemia or PD (Daviaud et al., 2013). Striatal–mesencephalon and cortex–striatum–mesencephalon co-cultures were developed to study tyrosine hydroxylase (TH)-positive neurons, being TH the rate-limiting enzyme in dopamine synthesis (Ostergaard et al., 1990). Those co-cultures maintained brain morphological and electrophysiological characteristics but did not mimic PD hallmarks (Plenz and Kitai, 1996). In order to better simulate *in vivo* models in which nigrostriatal degeneration is caused by dopaminergic neurotoxin injection, organotypic slices were next treated or injected with rotenone (Stahl et al., 2009) or 6-hydroxydopamine (6-OHDA) (Cavaliere et al., 2010). However, as in animal models, 6-OHDA led to neuronal death mediated by oxidative stress inducing selective dopaminergic neuron degeneration but did not produce extra-nigral pathology or Lewy body-like inclusions (Schober, 2004; Cavaliere and Matute, 2011). More recently, whole rat brain sagittal organotypic slices were developed which maintained dopaminergic and cholinergic neurons, as well as a complex capillary network and long nerve fibers (Ullrich et al., 2011). In the same way, sagittal sections of mouse brain were performed. These slices maintained the integrity of neuronal pathways during 3 weeks in culture and were immersed in 6-OHDA to induce a dopaminergic pathway degeneration (Kearns et al., 2006).

In the present study, we developed a sagittal organotypic culture model that includes all the areas involved in the nigrostriatal pathway in a unique slice without using neurotoxins to induce the dopaminergic lesion. The goal was to induce progressive nigrostriatal degeneration in a single step by mechanical transection of the medial forebrain bundle (MFB) while preparing the slices, in order to obtain a simple reproducible PD ex vivo model. We next studied slice survival while cultured with different media to maximize slice viability. The dopaminergic nigrostriatal pathway whose fibers run within the MFB was characterized by immunostaining against TH. Furthermore, progressive nigrostriatal degeneration was monitored combining two innovative approaches, magnetic resonance imaging (MRI) and mass spectrometry, rarely used for this type of study in organotypic slices. Fractional anisotropy (FA) measurements by diffusion tensor magnetic resonance imaging (DT-RMI) were used to follow and characterize MFB fiber degeneration in the organotypic slices. Indeed, DT-RMI provides quantitative information on water diffusion which can be used to evaluate the biological tissue properties. Furthermore, a liquid chromatography–mass spectrometry (LC–MS/MS) method to quantify striatal dopamine content directly in organotypic tissue was developed in this study. This new ex vivo PD model may represent a promising tool to refine the evaluation of new therapeutic approaches as cell therapy or tissue engineering, among others.

EXPERIMENTAL PROCEDURES

Preparation of nigrostriatal organotypic slices

Animal care and use were in strict accordance with the regulations of the French Ministry of Agriculture and all animal procedures were approved by animal experimentation ethics committee of Pays de la Loire. Every effort was made to minimize animal suffering and the number of animals used.

Timed pregnant Sprague–Dawley rats were purchased from Janvier (Saint Berthevin, France) or from SCAHU (Service commun d'animalerie hospitalo-universitaire, Angers University, France). Postnatal 6–8-day-old pups were used to prepare organotypic slices according to the Stoppini method (Stoppini et al., 1991) with modifications. Pups were anesthetized by an intraperitoneal injection of 80 mg/kg of ketamine (Clorketam 1000, Vetoquirol, Lure, France) and 10 mg/kg of xylazine (Rompum 2%, Bayar Health Care, Kiel, Germany). Animals were sacrificed, brains were rapidly dissected and cerebral hemispheres were separated and glued onto the chuck of a water-cooled vibratome (Motorized Advance Vibroslice MA752, Campdem Instruments, Loughborough, UK) to be sagittally cut. Under aseptic conditions, 400-μm slices were cut in different configurations in order to obtain a progressive degeneration of the nigrostriatal pathway. Finally, sagittal sections were cut alongside of the midline, with a 14-degree angle of the razor blade, and placed in sterile ice-cold Gray's Salt Balanced Solution (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 6.5 mg/L of glucose and antibiotics (100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.25 μg/ml amphotericin B) (Sigma–Aldrich, St. Louis, MO, USA) for 1 h. Typically, about 10 slices can be obtained per hemisphere. The first 2–3 slices and the two last brain slices did not contain the three main areas involved in the pathology and were discarded. Three to four slices per hemisphere were next transferred to 30-mm diameter semiporous membrane inserts (Millicell-CM, Millipore, Bedford, MA, USA) within a 6-well plate and put in culture at 37 °C, 5% CO_2. A total of about 30 rat pups and about 120 organotypic slices were necessary to perform the whole characterization. For each condition, a minimum of three slices taken from three different rat pups were used.

Nigrostriatal organotypic slice culture

Slices were cultured at the air–liquid interface and maintained using two different protocols. In the first protocol the slices were cultured during 16 days in a