Neurobiology of Disease 62 (2014) 307–312

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Abnormal structure-specific peptide transmission and processing in a primate model of Parkinson's disease and L-DOPA-induced dyskinesia

Mathieu Bourdenx [a,b,1], Anna Nilsson [e,1], Henrik Wadensten [e], Maria Fälth [e], Qin Li [c,d], Alan R. Crossman [c], Per E. Andrén [e,2], Erwan Bezard [a,b,c,d,*,2]

[a] Université de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, Bordeaux, France
[b] CNRS, Institut des Maladies Neurodégénératives, UMR 5293, Bordeaux, France
[c] Motac Neuroscience, Manchester, UK
[d] Institute of Laboratory Animal Sciences, China Academy of Medical Sciences, Beijing, China
[e] Department of Pharmaceutical Biosciences, Biomolecular Imaging and Proteomics, Uppsala University, Box 591, SE-75124 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 14 September 2013
Revised 7 October 2013
Accepted 10 October 2013
Available online 19 October 2013

Keywords:
Parkinson's disease
Mass spectrometry
Peptides
L-DOPA-induced dyskinesia
Primate
MPTP

ABSTRACT

A role for enhanced peptidergic transmission, either opioidergic or not, has been proposed for the generation of L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia (LID) on the basis of in situ hybridization studies showing that striatal peptidergic precursor expression consistently correlates with LID severity. Few studies, however, have focused on the actual peptides derived from these precursors. We used mass-spectrometry to study peptide profiles in the putamen and globus pallidus (internalis and externalis) collected from 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine treated macaque monkeys, acutely or chronically treated with L-DOPA. We identified that parkinsonian and dyskinetic states are associated with an abnormal production of proenkephalin-, prodynorphin- and protachykinin-1-derived peptides in both segments of the globus pallidus. Moreover, we report that peptidergic processing is dopamine-state dependent and highly structure-specific, possibly explaining the failure of previous clinical trials attempting to rectify abnormal peptidergic transmission.

Introduction

L-3,4-dihydroxyphenylalanine (L-DOPA) remains the most effective treatment for Parkinson's disease (PD) motor symptoms. However, L-DOPA-induced dyskinesia (LID) represents a major complication of treatment for PD. Among several mechanisms, a role for enhanced peptidergic transmission, either opioidergic or not, has been proposed for the generation of LID on the basis of in situ hybridization studies showing that striatal peptidergic precursor expression consistently correlates with LID severity (Aubert et al., 2007; Cenci et al., 1998; Henry et al., 2003; Tel et al., 2002). Parkinsonian and dyskinetic states have been associated with different patterns of expression of precursors of the peptides. Parkinsonism is associated with increased expression of the opioid precursor proenkephalin (PENK) messenger RNA (mRNA) in striatal neurons projecting to the globus pallidus in rodents (globus pallidus externalis (GPe) in primates) and a decreased prodynorphin (PDYN) mRNA expression in striatal neurons projecting to the substantia nigra pars reticulata in rodents and primates and the globus pallidus internalis (GPi) in primates (Aubert et al., 2007; Gerfen et al., 1990; Henry et al., 1999; Morissette et al., 1999; Nisbet et al., 1995; Quik et al., 2002). In the dyskinetic state, expression of PDYN mRNA is increased whereas PENK mRNA is unchanged versus controls, at least when the tissue was taken from animals killed at the peak of dyskinesia severity (Aubert et al., 2007).

Few studies have, however, focused on the actual peptides being processed from these precursors. We thus studied, using quantitative mass spectrometry, the peptidergic profiles of the putamen, GPe and GPi in parkinsonian and dyskinetic states using tissue collected from control and 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine (MPTP)-treated macaque monkeys, acutely or chronically treated with L-DOPA (Fernagut et al., 2010; Porras et al., 2012; Santini et al., 2010). The chronically L-DOPA-treated parkinsonian monkeys were separated into non-dyskinetic and dyskinetic groups.

Materials and methods

Ethical statement

All experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC)

* Corresponding author at: Institute of Neurodegenerative Diseases, Université Bordeaux Segalen, 146 rue Léo Saignat, 33076 Bordeaux cedex, France. Fax: +33 556986182.
E-mail address: erwan.bezard@u-bordeaux2.fr (E. Bezard).
Available online on ScienceDirect (www.sciencedirect.com).
[1] These authors contributed equally to this work.
[2] PEA and EB should be both considered as senior authors with equal contribution.

0969-9961/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nbd.2013.10.016

revised in 2010 (2010/63/EU) for care of laboratory animals in an AAALAC-accredited facility following acceptance of study design by the Institute of Lab Animal Science (Chinese Academy of Science, Beijing, China) IACUC for non-human primate experiments. A skilled veterinarian supervised animal care and maintenance.

Animal experimental protocol

Experiments were conducted using tissue from a previously published brain bank (Fernagut et al., 2010; Porras et al., 2012; Santini et al., 2010). Briefly, thirty female rhesus monkeys (*Macaca mulatta*, Xierxing, PR of China; mean of age = 5 ± 1 years; mean weight = 5.3 ± 0.8 kg) were randomly assigned to a particular treatment group (n = 6 per group) (Fig. 1A). Six animals were kept untreated as controls (Ctrl). The remaining twenty-four parkinsonian animals received daily MPTP (0.2 mg/kg, i.v., Sigma, St Louis, MO) according to our previously published protocol (Bezard et al., 1997, 2001a,b). Following stabilization of the MPTP-induced syndrome, animals received either saline (MPTP), a single dose of 20 mg/kg of L-DOPA p.o. (Acute LD) or this dose twice a day for three months (LD nonDysk/Dysk). Animal behavior was assessed as previously published (Ahmed et al., 2010; Fasano et al., 2010; Fernagut et al., 2010; Munoz et al., 2008; Porras et al., 2012). The degree of parkinsonism was assessed using a validated macaque clinical scale (Imbert et al., 2000). The severity of dyskinesia was rated using the Dyskinesia Disability Scale (Ahmed et al., 2010; Fasano et al., 2010; Fernagut et al., 2010; Fox et al., 2012; Munoz et al., 2008; Porras et al., 2012). All animals were killed by sodium pentobarbital overdose (150 mg/kg, i.v.) 1 h after the last dose of vehicle or L-DOPA. Brains were removed quickly after death and immediately frozen by immersion in isopentane (−45 °C) and stored at −80 °C.

Peptide extraction and sample preparation

Fresh frozen brains were sectioned in a Leica CM3050S cryostat (Leica Microsystems, Wetzlar, Germany) at −20 °C. Tissue punches of the putamen, GPe and GPi were collected from 300 μm-thick sections and stored at −80 °C until further use. The peptidergic fraction of samples was extracted as previously described (Nilsson et al., 2009; Svensson et al., 2003). Briefly, frozen brain samples were heat stabilized using irradiation at 95 °C (Stabilizor T1, Denator AB, Gothenburg, Sweden) (Svensson et al., 2009), transferred to low-retention Eppendorf tubes, weighted and suspended in pre-chilled extraction solution (7.5 μL of 0.25% acetic acid/mg tissue) and homogenized by sonication. Each sample suspension was then centrifuged and the supernatant was transferred to 10 kDa cut-off spin filters (YM-10, Millipore, Bedford, MA, USA) and centrifuged (Nilsson et al., 2009). The resulting filtrate was frozen at −80 °C until analysis.

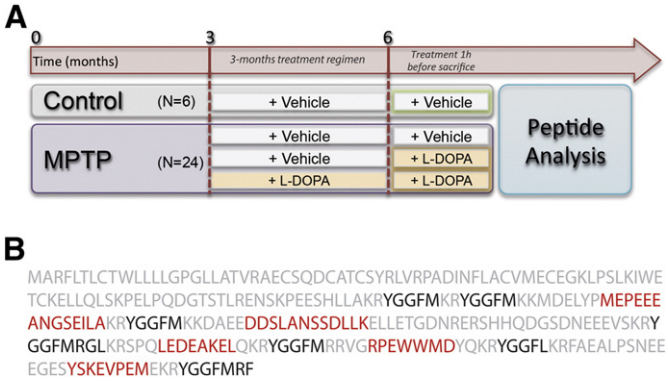

Fig. 1. A, Experimental flowchart illustrating study design and treatments. MPTP L-DOPA group comprises both dyskinetic and nondyskinetic animals B, Classic and new peptides processed from PENK precursor. The full-length peptide is in gray whereas the classic peptides are highlighted in black and the newly identified shared-motifs are highlighted in red.

Quantitation and identification of peptides by mass spectrometry

For quantitative mass spectrometry analysis, the samples were run in a restricted randomized block design (Kultima et al., 2009). Thus, one biological sample from each treatment group was randomly selected and consecutively analyzed (as one block of samples). This procedure was repeated until all samples were analyzed. Prior to each block of samples, a blank run (0.25% v/v aqueous acetic acid) was performed and a reference sample of pooled material was analyzed for internal quality control. An aliquot (5 μL) of the peptide filtrate was obtained from each animal and analyzed on a nano-liquid chromatography system (Ettan MDLC, GE Healthcare, Uppsala) using a nano-electrospray ionization interface coupled to an Q-Tof-2 (Waters, Manchester, UK) or a linear ion trap (LTQ, Thermo Electron, San Jose, CA) mass spectrometer for quantitation and identification, respectively. The label-free quantitation of relative peptide levels, normalization, data analysis, and the peptide identification was conducted as described previously (Kultima et al., 2009; Nilsson et al., 2009). This study was directed to search for peptide structures derived from the PENK, PDYN and the protachykinin-1 (TKN-1) systems, which have been correlated to different patterns of expression in parkinsonian and dyskinetic states.

Statistical analysis: The statistical analysis was performed in statistical programming language R using the Bioconductor package maanova (Gentleman et al., 2004; Wu et al., 2007). Linear regression normalization was done using all the detected peaks as previously described (Kultima et al., 2009; Nilsson et al., 2009). After normalization only peaks that were assigned a peptide sequence and were detected in at least two samples within a treatment group were included for further analyses. Student's *t*-test was used to identify significantly de-regulated peptides between two treatment groups, missing values were excluded. The level of statistical significance was set at $p < 0.05$. The p-values were not corrected for multiple testing since the number of test perform here was rather small, ranging from 75 to 200 depending on the brain region.

Results

Identification of peptides

Several peptides were identified from PENK, PDYN and TKN-1 precursors (Fig. 2). We identified 20 peptides deriving from PENK precursor showing significant changes in at least one comparison (Tables 1 and 2). As expected, we identified Met-Enkephalin (Met-Enk–YGGFM) and Leu-Enkephalin (Leu-Enk–YGGFL). We also identified two peptides which were C-terminally extended Met-Enk such as Met-Enk-R-G-L and Met-Enk-R-F. Peptides mentioned as 'others' are peptides deriving from PENK precursor but that did not content the Met-Enk motif (YGGFM). These 'other' peptides can be distinguished in five groups according to a shared-motif: MEPEEEANGSEILA (PENK 121–134), DDSLANSSDLLK (PENK 148–159), LEDEAKEL (PENK 200–208), RPEWWMDYQ (PENK 221–227), YSKEVPEM (252–259).

Deriving from both PENK and PDYN, Leu-Enk–YGGFL was identified in the putamen nucleus. Dynorphin A (DynA) was identified by its C-terminal (DynA 10–17) sequence: PKLKWDNQ. Substance P (RPKPQQFFGLM) and Neurokinin A (HKTDSFVGLM) deriving from TKN-1 precursor were also identified.

PENK, PDYN and TKN-1 peptide profiles

The parkinsonian state is associated with an increase ($p < 0.05$) in PENK-deriving peptides and Leu-Enk (which can derived from both PENK and PDYN) in the putamen and the GPe (Tables 1 and 2, Fig. 2). Other PENK-derived peptides were significantly decreased in GPe and