Experimental Neurology 226 (2010) 207–217

Contents lists available at ScienceDirect

Experimental Neurology

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

Decreased glutamic acid decarboxylase mRNA expression in prefrontal cortex in Parkinson's disease

Amélie C. Lanoue [a], Alexandra Dumitriu [b], Richard H. Myers [b], Jean-Jacques Soghomonian [a,*]

[a] Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts, 02118, USA
[b] Department of Neurology, Boston University School of Medicine, Boston, Massachusetts, 02118, USA

ARTICLE INFO

Article history:
Received 9 April 2010
Revised 27 August 2010
Accepted 1 September 2010
Available online 9 September 2010

Keywords:
GABA
Parkinson's disease
Prefrontal cortex
Glutamic acid decarboxylase
mRNA

ABSTRACT

Parkinson's disease (PD) patients typically suffer from motor disorders but mild to severe cognitive deficits can also be present. Neuropathology of PD primarily involves loss of dopaminergic neurons in the substantia nigra, pars compacta, although more widespread pathology from the brainstem to the cerebral cortex occurs at different stages of the disease. Cognitive deficits in PD are thought to involve the cerebral cortex, and imaging studies have identified the dorsolateral prefrontal cortex (DLPFC) as a possible site for some of the symptoms. GABAergic neurons in the cerebral cortex play a key role in the modulation of pyramidal neurons and alterations in muscimol binding to $GABA_A$ receptors have been reported in Brodmann area 9 (BA9) of the prefrontal cortex in PD patients (Nishino et al., 1988). In order to further assess the likelihood that GABAergic activity is altered in the prefrontal cortex in PD, gene expression of the 67 kilodalton isoform of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD67 encoded by the *GAD1* gene), was examined in BA9 of post-mortem brains from 19 patients and 20 controls using isotopic *in situ* hybridization histochemistry. GAD67 mRNA labeling was examined and quantified on X-ray films and emulsion radioautographs. We show that GAD67 mRNA labeling is significantly lower in PD compared to control cases. Analysis of emulsion radioautographs indicates that GAD67 mRNA labeling is decreased in individual neurons and is not paralleled by a decrease in the number of GAD67 mRNA-labeled neurons. Analysis of expression data from a microarray study performed in 29 control and 33 PD samples from BA9 confirms that GAD67 expression is decreased in PD. Another finding from the microarray study is a negative relationship between GAD67 mRNA expression and age at death. Altogether, the results support the possibility that GABAergic neurotransmission is impaired in the DLPFC in PD, an effect that may be involved in some of the behavioral deficits associated with the disease.

Parkinson's disease (PD) is primarily characterized by motor symptoms such as tremor, rigidity and bradykinesia, but it is well recognized that mild to severe cognitive dysfunction is present even in the early stages of the disease, significantly affecting quality of life (Brown and Marsden, 1990; Dubois and Pillon, 1997; Karlsen et al., 1998). Dementia will most often be seen in later stages of the disease (Mori, 2005). Cognitive deficits in PD include deficits in executive functions such as working memory, planning, sequence learning and visuomotor processing, functions that are classically attributed to the dorsolateral prefrontal cortex (DLPFC) (Goldman-Rakic, 1995; Fuster, 2000). The DLPFC includes Brodmann area 46 (BA46) and 9 (BA9) (e.g. Petrides, 2000). Imaging studies have documented abnormalities in the activation of the DLPFC in PD patients. For instance, regional blood flow is decreased in the DLPFC of PD patients compared to controls (Kikuchi et al., 2001) and a decreased fMRI signal was detected in the right and left DLPFC in cognitively impaired patients compared to cognitively unimpaired PD patients (Lewis et al., 2003). Activation of the DLPFC was also associated with motor sequence learning (Nakamura et al., 2001) or target retrieval (Carbon et al., 2003) in control and PD patients. Furthermore, deep-brain stimulation (DBS) of the subthalamic nucleus (STN) in PD patients alters regional blood flow (Sestini et al., 2002) or the fMRI signal in BA9/10 (Stefurak et al., 2003). Increased cerebral blood flow in the DLPFC induced by DBS of the STN correlates with DBS-induced decreased performance on the spatial delayed response task, a test of working memory (Campbell et al., 2008). It is therefore possible that altered activity of the DLPFC is involved in cognitive deficits in PD. Although dopamine, serotonin and acetylcholine in the DLPFC have been involved in cognitive deficits (e.g. Brooks and Piccini, 2006; Cools, 2006), the nature and mechanisms of neurochemical imbalances that could be associated with cognitive dysfunction in PD are still poorly documented.

* Corresponding author. Department of Anatomy and Neurobiology, Boston University School of Medicine, 715 Albany Street, Room L1004, Boston, MA 02118, USA. Fax: +1 617 638 4216.
E-mail address: jjsogho@bu.edu (J.-J. Soghomonian).

0014-4886/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2010.09.001

GABAergic interneurons play a key role in the circuitry of the cerebral cortex and the DLPFC, where they exert a major control on the activity of pyramidal neurons. Earlier biochemical studies assessed the impact of PD on the activity of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD) (Monfort et al., 1985; Nishino et al., 1988) or GABA levels (Gerlach et al., 1996) in the prefrontal cortex, but no significant effect was reported. However, decreased binding of muscimol to $GABA_A$ receptors was shown in BA9 (Nishino et al., 1988) indicating that altered GABAergic activity in this area might be a neuropathological feature of PD. Two different isoforms of GAD known as GAD67 and GAD65, encoded by two genes *GAD1* and *GAD2*, are involved in the biosynthesis of GABA in GABAergic neurons in the adult brain (Erlander et al., 1991; reviewed in Soghomonian and Martin, 1998). The GAD67 isoform is known to provide most levels of neuronal GABA *in vivo* (Asada et al., 1997) and is highly expressed in the cerebral cortex (Feldblum et al., 1993; Esclapez et al., 1994; Hendrickson et al., 1994). In order to further assess the possibility that the activity of GABAergic neurons in the prefrontal cortex is altered in PD, we used *in situ* hybridization histochemistry to detect and measure GAD67 mRNA labeling in BA9 from control and PD brains. Additionally, we analyzed microarray expression data to contrast GAD67 mRNA levels in control and PD samples of BA9.

Subjects and methods

Human subjects and tissue sectioning

For the *in situ* hybridization studies, samples of BA9 were obtained from the Harvard Brain Tissue Resource Center (HBTRC; McLean Hospital, Belmont, Massachusetts) and the Sun Health Research Institute (SHRI; Sun City, Arizona) (Table 1). For the microarray study, samples from HBTRC, SHRI, and the Human Brain and Spinal Fluid Resource Center (HBSFRC) VA West Los Angeles Healthcare Center, California, were used (Table 2). All subjects were male. All subjects included in the PD group had a clinical diagnostic of PD and a pathological diagnostic of PD determined by the presence of Lewy bodies in the substantia nigra, pars compacta, but cognitive measures were not available. The pH of all samples used in the microarray, and

Table 1
Human subjects data used for the *in situ* hybridization experiments.

Gender	Age at death (years)	PMI (hours)	pH	Disease duration (years)	Cause of death	Dementia status
Harvard Brain Tissue Resource Center (HBTRC)						
Control cases						
Male*	66	18.7	6.75	N/A	Myocardial infarction	N/A
Male*	69	15.3	7.323	N/A	Respiratory failure; chronic obstructive pulmonary disease	N/A
Male	40	16.6	6.5925	N/A	Cardiac	N/A
Male	106	21	6.7075	N/A	Congestive heart failure; acute renal failure; myocardial infarction	N/A
Male	44	28.17	6.965	N/A	Cardiac arrest	N/A
Male	57	24.42	6.7125	N/A	Myocardial infarction	N/A
Male	43	14.68	6.985	N/A	Myocardial infarction	N/A
Male	52	22.95	6.3725	N/A	Heart attack	N/A
PD cases						
Male	75	29.6	6.235	14	Aspiration pneumonia	Yes
Male	68	18.4	N/A	6	Myocardial infarction	Yes
Male	68	22.87	6.8475	5	Cardiac arrest	N/A
Male	79	11.77	6.415	13	Aspiration pneumonia	Yes
Male	75	19.42	5.885	18	Dementia; PD	Yes
Male*	74	15.15	6.67	N/A	End stage PD; bladder infection	N/A
Male*	89	30.75	6.675	17	End stage PD	No
Male*	66	11.21	6.735	11	PD	No
Sun Health Research Institute (SHRI)						
Control cases						
Male*	86	3	6.435	N/A	Respiratory failure	N/A
Male*	91	1.5	6.29	N/A	Metastatic bladder cancer	N/A
Male	73	2.5	6.845	N/A	Acute myeloid leukemia due to myelodysplastic syndrome	N/A
Male*	97	1.5	7.137	N/A	Metastatic colon cancer	N/A
Male	74	2.5	N/A	N/A	Cardiac and/or respiratory failure	N/A
Male	69	2	N/A	N/A	Prostate cancer	No
Male*	79	2	6.915	N/A	Cardiac and/or respiratory failure	N/A
Male	76	2.5	N/A	N/A	Pancreatic cancer	N/A
Male	78	2.66	N/A	N/A	Cardiac and/or respiratory failure	N/A
Male*	63	1.5	6.603	N/A	Acute intracerebral hemorrhage	N/A
Male	86	2.5	7.1075	N/A	Congestive heart failure; ischemic cardiomyopathy	N/A
Male	78	1.66	N/A	N/A	Lung cancer; heart failure	N/A
PD cases						
Male*	85	4	6.537	15	Ruptured abdominal aortic aneurysm	No
Male	85	2.16	6.7275	6	Lung cancer	No
Male*	77	1.66	6.438	13	PD	N/A
Male	72	2	6.5	10	Brain cancer — glioblastoma	N/A
Male	83	2	6.58	7	Pneumonia; hypertension; vascular dementia	Yes
Male*	72	3.5	6.72	17	Possible cerebrovascular accident; end stage PD	Yes
Male*	77	1.16	6.593	22	End stage PD; complications from fall	No
Male*	83	2.16	6.838	4	End stage PD	Yes
Male*	80	2.25	6.65	25	End stage PD; inanition	No
Male*	84	2.5	6.47	4	N/A	Yes
Male*	88	2	6.71	3	End stage chronic obstructive pulmonary disease	Yes

* Indicates subjects also used in the microarray study.