Neurobiology of Disease 45 (2012) 763–773

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

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

Emerging dysfunctions consequent to combined monoaminergic depletions in parkinsonism

Claire Delaville [a], Jonathan Chetrit [a], Khaled Abdallah [a], Stéphanie Morin [a], Laura Cardoit [a], Philippe De Deurwaerdère [a], Abdelhamid Benazzouz [a,b,*]

[a] Université Bordeaux Segalen, Centre National de la Recherche Scientifique (CNRS UMR 5293), Neurodegenerative Diseases Institute, Group Monoamines, DBS and Parkinson's disease, 146 rue Léo-Saignat, 33076 Bordeaux Cedex, France
[b] Centre Hospitalier Universitaire de Bordeaux, Place Amélie Raba-Léon, 33000 Bordeaux, France

ARTICLE INFO

Article history:
Received 31 August 2011
Revised 19 October 2011
Accepted 28 October 2011
Available online 4 November 2011

Keywords:
Parkinson's disease
Dopamine
Noradrenaline
Serotonin
Monoamine depletion
Motor symptoms
Non-motor symptoms
Single unit microrecording
Subthalamic nucleus

ABSTRACT

The loss of dopamine (DA) neurons has been the pathophysiological focus of the devastating conditions of Parkinson's disease, but depletion of DA alone in animal models has failed to simultaneously elicit both the motor and non-motor deficits of PD. The present study aimed to investigate, in rats, the respective role of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) depletions on motor and non-motor behaviors and on subthalamic (STN) neuronal activity. We show that NA or DA depletion significantly decreased locomotor activity and enhanced the proportion of bursty and irregular STN neurons. Anxiety-like states required DA depletion plus the depletion of 5-HT or NA. Anhedonia and "depressive-like" behavior emerged only from the combined depletion of all three monoamines, an effect paralleled by an increase in the firing rate and the proportion of bursty and irregular STN neurons. Here, we provide evidence for the exacerbation of behavioral deficits when NA and/or 5-HT depletions are combined with DA depletion, bringing new insight into the combined roles of the three monoamines in PD.

Introduction

Parkinson's disease is a neurological disorder characterized by the manifestation of motor symptoms, attributed to the degeneration of dopamine (DA) neurons of the substantia nigra pars compacta (SNc) (Ehringer and Hornykiewicz, 1960). Although the motor symptoms of PD are well defined, the non-motor features, such as depression, anhedonia and anxiety, are under-studied and, consequently, under-treated.

Despite the focus on DA, PD is a multi-system disorder characterized also by the loss of noradrenaline (NA) neurons of the locus coeruleus (Bertrand et al., 1997; Fornai et al., 2007) and serotonin (5-HT) cells of the dorsal raphe (Kish, 2003). Although NA and 5-HT depletions have been suggested as other landmarks of the disease, a specific role for each neurotransmitter in the pathophysiology of PD is still not clearly determined. NA and 5-HT are widely recognized in the development of depression and anxiety both of which have been reported in PD patients (Delaville et al., 2011; Halliday et al., 1990; Murai et al., 2001). On the other hand, total inhibition or destruction of the 5-HTergic system alone in the rat does not induce any "depressive-like" behavior or anxiety (Cervo et al., 1991; Wieland et al., 1990). Considering these findings together, we hypothesized that these symptoms could be a consequence of dysfunction of some combination of DAergic, NAergic and 5-HTergic pathways.

Many studies have identified the subthalamic nucleus (STN) as a basal ganglia nucleus playing a key role in the pathophysiology of PD. After DA depletion, STN neurons, which normally exhibit a tonic discharge pattern, become bursty in animal models of PD (Bergman et al., 1994; Ni et al., 2001). This pathological bursty pattern has also been reported in PD patients (Benazzouz et al., 2002; Hutchison et al., 1998). Moreover, the motor symptoms of PD are alleviated by either STN ablation (Bergman et al., 1990; Guridi and Obeso, 2001) or high frequency stimulation (Benabid et al., 2000; Benazzouz et al., 1993). In addition to its role in motor regulation, the STN plays a pivotal role in associative and limbic functions (Temel et al., 2005). Furthermore, within the basal ganglia, the STN is one of the structures most heavily innervated by NAergic (Boyajian et al., 1987; Canteras et al., 1990) and 5-HTergic (Steinbusch, 1981) terminals. Functionally, NAergic as well as 5-HTergic agents, can modulate STN neuronal activity with an impact on motor behavior in the rat (Belujon et al., 2007; De Deurwaerdere and Chesselet, 2000). From these studies, and in

* Corresponding author at: Université Bordeaux Segalen, 146 rue Léo-Saignat, 33076 Bordeaux Cedex, France. Fax: +33 556901421.
E-mail address: abdelhamid.benazzouz@u-bordeaux2.fr (A. Benazzouz).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2011.10.023

view of its important role in the basal ganglia and in PD, we hypothesized that defective NAergic and/or 5-HTergic transmission could influence the electrical activity of STN neurons in the context of PD.

Thus the present study aimed to investigate the effects of DA, NA and 5-HT depletions one by one or combined upon (i) motor and non-motor functions including locomotor activity, depressive-like behavior and anxiety, and (ii) on the electrical activity of STN neurons in the rat.

Material and methods

Animals

Adult male Wistar rats, weighing 280–380 g were used for behavioral and *in vivo* electrophysiological experiments. They were housed five per cage under artificial conditions of light (light/dark cycle, light on at 7:00 a.m.), temperature (24 °C), and humidity (45%) with food and water available *ad libitum*. All animal experiments were carried out in accordance with the European Communities Council Directive (*EU Directive 2010/63/EU*).

Monoamine depletion procedures

The present study was carried out on a total of 151 rats distributed in eight groups as summarized in Fig. 1. Each group was subdivided into subgroups to perform one or two behavioral tests. The number of animals per group appears in the figure legends.

Each rat received either 6-hydroxydopamine (6-OHDA-lesioned rats) or NaCl (0.9%; sham-lesioned rats) into the right medial forebrain bundle (MFB). Two weeks later, 6-OHDA-lesioned rats and sham-lesioned rats received intraperitoneal (i.p.) injection of N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) or NaCl (0.9%) followed, one week later, by two i.p. injections of 4-Chloro-L-phenylalanine (pCPA) or NaCl (0.9%) on two consecutive days. The rationale for waiting 2 weeks after 6-OHDA injection to deliver the NA- and 3 weeks for 5-HT-depleting drugs was based on the fact that the stable stage of behavioral deficits, as well as the pathological activity in basal ganglia nuclei, appears at least 2 weeks after the injection of 6-OHDA (Neve et al., 1982; Ni et al., 2001; Orieux et al., 2000). Furthermore, behavioral or electrophysiological experiments were then performed 4 weeks after 6-OHDA, 1 week after DSP-4 and/or the day following the last pCPA injection. Animals exposed to 2 or 3 depletions did not develop any weight loss and had a good general welfare.

6-OHDA, DSP-4 and pCPA were purchased from Sigma (Saint-Quentin Fallavier, France). NA depletion has been performed using DSP-4, a neurotoxin highly selective for NAergic fibers arising from the LC that does not affect other NAergic systems, such as the sympathetic nervous system (Fornai et al., 2001; Fritschy and Grzanna, 1989, 1991). DSP-4 was used at a dose of 50 mg/kg according to the work of Grzanna et al. (1989). It was dissolved in NaCl 0.9% immediately before use. pCPA, a selective inhibitor of 5-HT synthesis, was also used at a dose of 50 mg/kg during two successive days as previously determined in the laboratory (data not shown). 6-OHDA was stereotaxically injected into the MFB as previously described (Belujon et al., 2007). Thirty minutes prior to surgery, animals were given an i.p. injection of desipramine (25 mg/kg, Sigma) dissolved in 0.9% NaCl and injected in a volume of 5 ml/kg body weight. Desipramine was used in order to protect the NAergic system. Rats were then placed in a stereotaxic frame (Kopf, Unimecanique, France) under chloral hydrate anesthesia (400 mg/kg, i.p., Sigma). Each animal received a unilateral injection of 2.5 µl 6-OHDA (Sigma, 5 mg/ml in sterile NaCl, 0.9%) with 0.1% ascorbic acid into the right MFB at coordinates 2.8 mm posterior to bregma, 2 mm lateral to the midline and 8.4 mm below the skull according to the brain atlas of Paxinos and Watson (1996). 6-OHDA injection was made over a 5 min period using a 10 µl Hamilton microsyringe. At the end of each injection, the syringe needle was left in place for an additional 5 min and then withdrawn slowly to prevent reflux of the solution.

Evaluation of motor activity

Spontaneous motor activity (open-field)

Spontaneous horizontal motor activity was measured in an isolated room between 8:00 a.m. and 1:00 p.m. using a photoelectric actimeter (Actitrack, Panlab, S.L., Barcelona, Spain), as previously described (Chetrit et al., 2009).

Evaluation of catalepsy scores (bar test)

The degree of catalepsy resulting from monoamine depletions was assessed using the bar tests as previously reported (Chetrit et al., 2009). It consisted of positioning the rat's forepaws on a horizontal bar (0.7 cm diameter) placed at 9 cm above the ground and measuring the latency for the forepaw contralateral to the 6-OHDA-lesioned

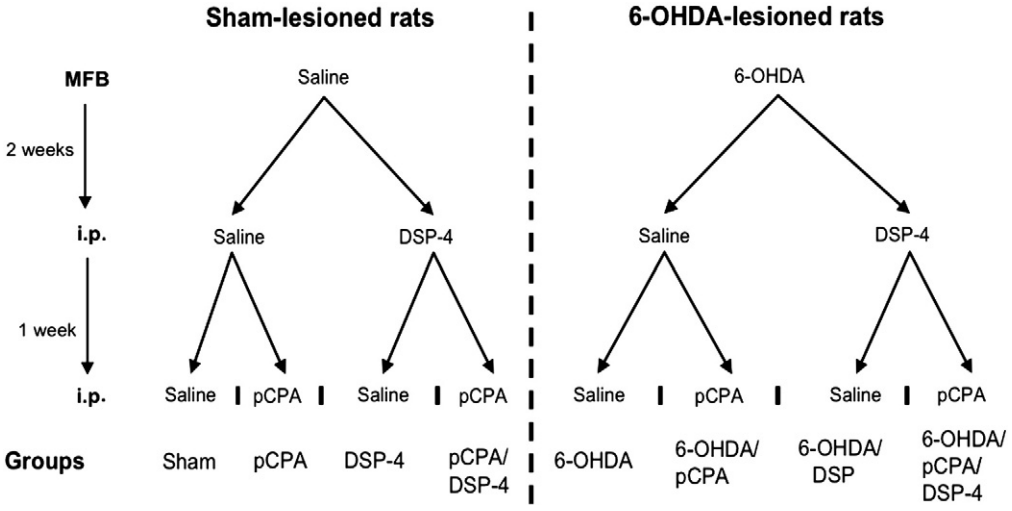

Fig. 1. Schematic presentation of different groups of drug-treated animals and their respective controls. MFB: medial forebrain bundle; i.p.: intra-peritoneal injection. Sham: rats treated with 0.9% NaCl (n = 17); pCPA: rats treated with 4-Chloro-L-phenylalanine (n = 19); DSP-4: rats treated with N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (n = 10), pCPA/DSP-4: n = 21; 6-OHDA: rats treated with stereotactic injection of 6-hydroxydopamine (6-OHDA) into the MFB (n = 8), 6-OHDA/pCPA: n = 23, 6-OHDA/DSP-4: n = 13, 6-OHDA/pCPA/DSP-4: n = 19.