Neuroscience 210 (2012) 375–383

LEAD INTOXICATION INDUCES NORADRENALINE DEPLETION, MOTOR NONMOTOR DISABILITIES, AND CHANGES IN THE FIRING PATTERN OF SUBTHALAMIC NUCLEUS NEURONS

M. SABBAR,[a,b] C. DELAVILLE,[a,c] P. DE DEURWAERDÈRE,[a,c] A. BENAZZOUZ[a,c1]* AND N. LAKHDAR-GHAZAL[b1]

[a]*Univ. de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, F-33000 Bordeaux, France*

[b]*Université Mohamed V-Agdal, Faculté des Sciences, Equipe Rythmes Biologiques et Environnement, Rabat, Morocco*

[c]*CNRS, Institut des Maladies Neurodégénératives, UMR 5293, F-33000 Bordeaux, France*

Abstract—Lead intoxication has been suggested as a high risk factor for the development of Parkinson disease. However, its impact on motor and nonmotor functions and the mechanism by which it can be involved in the disease are still unclear. In the present study, we studied the effects of lead intoxication on the following: (1) locomotor activity using an open field actimeter and motor coordination using the rotarod test, (2) anxiety behavior using the elevated plus maze, (3) "depression-like" behavior using sucrose preference test, and (4) subthalamic nucleus (STN) neuronal activity using extracellular single unit recordings. Male Sprague–Dawley rats were treated once a day with lead acetate or sodium acetate (20 mg/kg/d i.p.) during 3 weeks. The tissue content of monoamines was used to determine alteration of these systems at the end of experiments. Results show that lead significantly reduced exploratory activity, locomotor activity and the time spent on the rotarod bar. Furthermore, lead induced anxiety but not "depressive-like" behavior. The electrophysiological results show that lead altered the discharge pattern of STN neurons with an increase in the number of bursting and irregular cells without affecting the firing rate. Moreover, lead intoxication resulted in a decrease of tissue noradrenaline content without any change in the levels of dopamine and serotonin. Together, these results show for the first time that lead intoxication resulted in motor and nonmotor behavioral changes paralleled by noradrenaline depletion and changes in the firing activity of STN neurons, providing evidence consistent with the induction of atypical parkinsonian-like deficits.

Key words: lead, Parkinson disease, subthalamic nucleus, noradrenaline, electrophysiology.

Heavy metals are well known for their neurotoxic effects inducing neurological disorders in humans (Tavakoli-Nezhad et al., 2001; Perl, 2006; Fleece and Robinson, 2007) and animals (Crapper et al., 1973; Michaelson and Sauerhoff, 1974; Rice, 2000; Shaw and Petrik, 2009). Lead is a major environmental pollutant, and its primary target is the central nervous system (Kishi et al., 1983; Rodrigues et al., 1996). After exposure, lead is stored in the bone by replacing calcium, which is released into the bloodstream and circulates throughout the body. In the brain, it diffuses easily across the blood brain barrier and causes variable changes in several neurotransmitter systems. Lead interferes predominantly with the most common excitatory neurotransmitter in the brain, glutamate, which is critical for learning (Cory-Slechta, 1995) and intracellular oxidative stress (Quinlan et al., 1988; Bradbury and Deane, 1993; Sandhir et al., 1994; Reckziegel et al., 2011). Several epidemiologic studies support an association between parkinsonism and exposure to heavy metals, suggesting that lead exposure can be a high-risk factor for development of the disease (Gorell et al., 1997; Coon et al., 2006). These authors reported a 2-fold increase in the risk of Parkinson disease among workers with occupational exposure to lead. However, there is no evidence for a direct link between lead exposure and the manifestation of parkinsonian-like symptoms.

Parkinson disease is a neurological disorder characterized by motor and nonmotor disabilities. Motor symptoms are attributed to the degeneration of dopamine (DA) neurons of the substantia nigra pars compacta (Ehringer and Hornykiewicz, 1960) and nonmotor symptoms to the additional degeneration of noradrenaline (NA) neurons in the locus coeruleus and serotonin cells mainly from the dorsal raphe. Although the primary cause of cell death is unknown, the pathophysiology of the disease has been profoundly investigated during the past three decades. As a part of the basal ganglia network, the subthalamic nucleus (STN) has been identified as a key structure, playing a role in the pathophysiology of Parkinson disease. After DA depletion, STN neurons, which normally exhibit a tonic discharge pattern, become bursty in animal models of the disease (Bergman et al., 1994; Hassani et al., 1996; Ni et al., 2001; Belujon et al., 2007; Breit et al., 2007; Delaville et al., 2012a). This pathological bursty pattern has also been reported in parkinsonian patients (Hutchison et al., 1998; Benazzouz et al., 2002). In addition, the motor symptoms are alleviated by either STN lesion (Bergman et al., 1990) or high frequency electrical stimulation in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-monkeys (Benazzouz et

[1] These authors contributed equally.

*Correspondence to: A. Benazzouz, Institut des maladies Neurodégénératives (IMN), CNRS UMR 5293, Université Bordeaux Segalen, 146 Rue Léo-Saignat, 33076 Bordeaux Cedex, France. Tel.: +33-557-57-46-25; fax: +33-55-69-01-421.
E-mail address: abdelhamid.benazzouz@u-bordeaux2.fr (A. Benazzouz).

Abbreviations: COX, cytochrome oxidase; DA, dopamine; EPM, Elevated plus maze; HPLC, high performance liquid chromatography; NA, noradrenaline; STN, subthalamic nucleus.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
10.1016/j.neuroscience.2012.02.026

al., 1993, 1996) and parkinsonian patients (Limousin et al., 1998; Krack et al., 2003). We hypothesized that lead exposure could induce defective monoamine transmission resulting in a perturbation of the electrical activity of STN neurons, which can be, at least in part, at the origin of motor and nonmotor deficits.

Thus, the present study aimed to investigate the effects of lead exposure upon (i) motor and nonmotor functions including exploratory and locomotor activity, motor coordination, "depressive-like" behavior, and anxiety and on (ii) the electrical activity of STN neurons in the rat. Postmortem high performance liquid chromatography (HPLC) was used to control potential depletion of monoamines as follows: DA, NA, and serotonin.

EXPERIMENTAL PROCEDURES

Animals and lead treatment protocol

Twenty male Sprague–Dawley rats (PN40) were used for behavioral experiments. Animals were obtained from the "Centre d'Elevage Depré" (Centre d'Elevage Depré, Saint-Doulchard, France). Animals were maintained in a room with constant temperature (24 °C) and humidity (45%), exposed to a 12-h light/12-h dark cycle (light on at 7:00 AM) and had access to food and water *ad libitum*. All animal experiments were carried out in accordance with European Communities Council Directive 2010/63/UE, and all efforts were made to minimize the number of animals used and their suffering. All animal experiments were carried out in accordance with European Communities Council Directive 2010/63/UE, and all efforts were made to minimize the number of animals used and their suffering. Animals were divided into two groups (10 rats per group) as follows: lead-treated animals received daily i.p. injections of lead acetate (20 mg/kg) solution for 3 weeks, and the control animals received sodium acetate (20 mg/kg) in the same conditions. The dose of lead acetate was selected on the basis of a detailed literature search (Mehdizadeh et al., 2008). Moreover, Ito et al. (1985) have shown a nearly similar blood lead concentration in workers exposed to lead and in rats intoxicated by lead acetate at this dose. The animals' weights were monitored throughout the experiment.

The Fig. 1 summarizes the experimental design, with a time course of all the motor and nonmotor behavioral tests. After the last test, animals were used for electrophysiological recordings of STN neurons. Immediately after each electrophysiological session, the rats were sacrificed and the brains removed for postmortem biochemical and histochemical assessments.

Evaluation of exploratory and locomotor behaviors (open field)

Spontaneous horizontal and vertical (rearing) activities as well as stereotyped movements were measured using a photoelectric actimeter (Actitrack, Panlab, Barcelona, Spain), as previously described (Chetrit et al., 2009). Briefly, the apparatus consisted of a transparent cage equipped with photoelectric cells. Light beams detected movement, and the total locomotor activity of each rat was recorded over two successive sessions of 10 min each during 4 days in an isolated room between 8:00 AM and 2:00 PM. The test started the day following the last injection of lead acetate or sodium acetate. The two sessions of 10 min were used for data analysis. The first session was used to evaluate exploratory activity, and the second session evaluated the motor behavior of the animals.

Evaluation of motor coordination (rotarod)

To assess the effect of lead treatment on motor coordination, rats were trained to remain on a rotarod (Bioseb, *in vivo* Research Instruments, Spain), as previously described (Papp and Bal, 1987; Rozas et al., 1997). All rats underwent a 3-day training program on a 7-cm diameter rotarod. During the training period, each rat was placed on a horizontal rod rotating at a gradually increasing speed from 4 to 20 rotations per minute (rpm) for a maximum of 15 min by which time a steady baseline level of performance was attained. The day after training, the motor coordination was recorded for each animal during five trials. The latency to fall off the rotarod was recorded and the time limit was fixed at 3 min.

Evaluation of "depression-like" behavior or anhedonia

The sucrose preference test was used, as previously described (Delaville et al., 2012a). Rats were housed in individual cages with food and water *ad libitum*. Three days before the experiment onset, rats were housed in the presence of two bottles of water and the position of the bottles was randomly changed to prevent a place preference. On the test day, when the light off at 7:00 PM, preweighed water and 1% sucrose-containing bottles were placed on the home cage and rats were allowed to drink for 2 h. During these 2 h, the position of the bottles was changed four times. Water or sucrose absorption was measured by weighing the bottles before and after the test. Sucrose preference was calculated as follows:

$$100 \times [\text{sucrose intake (g)} / (\text{sucrose intake (g)} + \text{water intake (g)})]$$

Evaluation of anxiety

Animals were tested in the elevated plus maze (EPM) to assess anxiety-related behavior, as previously reported (Delaville et al., 2012a). The EPM consisted of two open arms (50-cm long×10-cm wide) and two enclosed arms (50-cm long×10-cm wide×38.5-cm high), with an open roof arranged around a central platform (10 ×10 cm^2). Arms of the maze form a cross, with two open arms being opposite each other. A camera was mounted 1.5 m above the EPM to record the frequency and duration of open and closed arm visits under red light. Animals were placed onto the central platform, facing one of the open arms. Each animal was allowed to explore the maze for 5 min. The time spent in the open and closed arms and the numbers of entries into the arms were taken in account when the animal entered an arm with all four paws. Animals were considered anxious when they spent less time in the open arms.

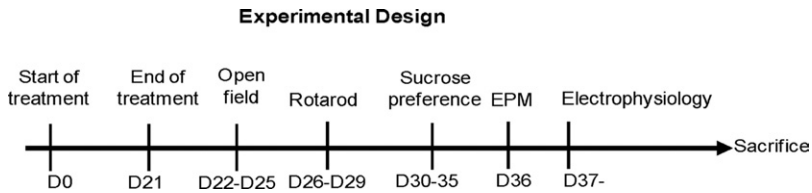

Fig. 1. Schematic presentation of the experimental design, with a time course of all the behavioral and electrophysiological tests.