Neuroscience 236 (2013) 289–297

INTRAPALLIDAL ADMINISTRATION OF 6-HYDROXYDOPAMINE MIMICS IN LARGE PART THE ELECTROPHYSIOLOGICAL AND BEHAVIORAL CONSEQUENCES OF MAJOR DOPAMINE DEPLETION IN THE RAT

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Abstract—In addition to GABA and glutamate innervations, the globus pallidus (GP) receives dopamine afferents from the *pars compacta* of the substantia nigra (SNc), and in turn, sends inhibitory GABAergic efferents to the subthalamic nucleus (STN) and the *pars reticulata* of the substantia nigra (SNr). Nevertheless, the role of dopamine in the modulation of these pallido-subthalamic and pallido-nigral projections is not known. The present study aimed to investigate the effects of intrapallidal injection of 6-hydroxydopamine (6-OHDA) on the electrical activity of STN and SNr neurons using *in vivo* extracellular single unit recordings in the rat and on motor behaviors, using the ''open field'' actimeter and the stepping test. We show that intrapallidal injection of 6-OHDA significantly decreased locomotor activity and contralateral paw use. Electrophysiological recordings show that 6-OHDA injection into GP significantly increased the number of bursty cells in the STN without changing the firing rate, while in the SNr neuronal firing rate decreased and the proportion of irregular cells increased. Our data provide evidence that intrapallidal injection of 6-OHDA resulted in motor deficits paralleled by changes in the firing activity of STN and SNr neurons, which mimic in large part those obtained after major dopamine depletion in the classical rat model of Parkinson's disease. They support the assumption that in addition to its action in the striatum, dopamine mediates its regulatory function at various levels of the basal ganglia circuitry, including the GP.

Key words: Parkinson's disease, dopamine, globus pallidus, subthalamic nucleus, substantia nigra *pars reticulata*, electrophysiology.

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Abbreviations: 6-OHDA, 6-hydroxydopamine; GP, globus pallidus; HPLC, high-performance liquid chromatography; PD, Parkinson's disease; SNc, *pars compacta* of substantia nigra; SNr, *pars reticulata* of substantia nigra; STN, subthalamic nucleus.

INTRODUCTION

Parkinson's disease (PD) is a neurological disorder characterized by the progressive degeneration of dopamine neurons in the *pars compacta* of the substantia nigra (SNc), resulting in a loss of dopamine in the striatum (Ehringer and Hornykiewicz, 1960). This discovery contributed to the development of dopamine replacement therapy in PD (Andén et al., 1970) and the understanding of the mechanisms by which dopamine modulates neuronal function in the striatum (for review, Nicola et al., 2000). Another contribution was the concept of the ''direct and indirect'' pathways model of basal ganglia circuitry (Albin et al., 1989; Alexander and Crutcher, 1990; Bergman et al., 1990), which was at the origin of the development of a new neurosurgical therapy of PD by subthalamic nucleus (STN) high-frequency stimulation (Benazzouz et al., 1993; Limousin et al., 1995). This basic model represented an oversimplification of the basal ganglia organization and many studies have led to its refinement for better understanding of how dopamine regulates the basal ganglia and how it contributes to the pathophysiology of PD (for review, Smith and Villalba, 2008).

While the striatum is by far the main target of SNc dopamine neurons, dopamine can mediate its regulatory function at various levels of the basal ganglia circuitry, including the globus pallidus (GP) and STN (Smith and Kieval, 2000; Smith and Villalba, 2008).

The GP and STN are two interconnected nuclei of the indirect pathway (Parent and Hazrati, 1995a). Although GP and STN neurons pattern each other's activity, the GP is considered as a structure controlling the electrical activity of STN neurons by means of its GABAergic projections (Hallworth and Bevan, 2005; Baufreton et al., 2009). In addition to massive GABAergic axon terminals from the striatum and glutamatergic afferents from the STN and the parafascicular nucleus of the thalamus (Kincaid et al., 1991; Mouroux et al., 1997), GP receives dopaminergic afferents from the SNc. These dopaminergic afferents are considered as collaterals of the nigrostriatal projections (François et al., 1999; Smith and Kieval, 2000; Bouali-Benazzouz et al., 2009) and specific nigro-pallidal projections (Jan et al., 2000; Smith and Kieval, 2000; Rommelfanger and Wichmann, 2010). Recent evidence suggests that dopamine modulates GP function and that dopamine receptor blockade or dopamine depletion at this site

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.01.043

contributes to motor deficits (Hauber and Lutz, 1999; Bouali-Benazzouz et al., 2009).

Dopamine in the GP controls both GABAergic and glutamatergic inputs *via* D1 and D2 receptor subtypes (Floran et al., 1990; Floran et al., 1997; Cooper and Stanford, 2001; Shin et al., 2003; Hernández et al., 2006). In turn, GP neurons send inhibitory GABAergic projections to the STN and the substantia nigra *pars reticulata* (SNr) (Parent and Hazrati, 1995b; Rommelfanger and Wichmann, 2010). Nevertheless, the impact of dopamine depletion in GP on the modulation of these pallido-subthalamic and pallido-nigral projections is not known.

The present study aimed to investigate the effects of intrapallidal injection of 6-hydroxydopamine (6-OHDA), on the electrical activity of STN and SNr neurons using *in vivo* extracellular single-unit recordings in the rat and on motor behavior, using the ''open field'' actimeter and the stepping test.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats, weighing 280–360 g, were used in this study. Animals were housed three per cage, kept under artificial conditions of light (12-h light/dark cycle, light on at 7:00 A.M.), temperature (24 °C), and humidity (45%) with food and water available *ad libitum*. All efforts were made to minimize the number of animals used and their suffering. Experiments were carried out in accordance with the European Communities Council Directive (*EU Directive 2010/63/EU*) and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

6-OHDA injection into the GP

As described previously (Bouali-Benazzouz et al., 2009), 30 min before surgery, animals were pretreated with desipramine (25 mg/kg, Sigma, France), dissolved in 0.9% sterile sodium chloride (NaCl) and injected intraperitoneally (i.p.) in order to protect noradrenergic pathways against 6-OHDA neurotoxicity. Rats then were placed in a stereotaxic frame (Kopf, Unimecanique, Paris, France) under xylazine (xylazine hydrochloride, 10 mg/kg, i.p, Sigma, France) and ketamine anesthesia (ketamine hydrochloride, 75 mg/kg, i.p., Sigma, France). Each animal received a unilateral injection of 2.5-µl 6-OHDA (5 mg/ml in sterile NaCl, 0.9%; Sigma, France) with 0.01% ascorbic acid into the right GP at coordinates 0.9 mm posterior to bregma, 3.0 mm lateral to the midline and 6.5 mm below the dura according to the brain atlas of Paxinos and Watson (1998). The 6-OHDA injection was made over a 5-min period using a cannula connected by polyethylene tubing to a 10-µl Hamilton microsyringe. At the end of each injection, the cannula was left in place for an additional 5 min to prevent reflux of the solution and to allow for toxin diffusion and then withdrawn slowly. For the sham group, vehicle (saline plus 0.01% ascorbic acid) was infused into the GP under the same conditions as for 6-OHDA.

Nineteen rats were used and distributed in two groups, 6-OHDA-lesioned group (n = 9 rats) and sham group (n = 10 rats). All these rats were tested for locomotor activity 4 weeks after surgery. Then, electrophysiological recordings were carried out in the STN (n = 5 sham and n = 5 6-OHDA rats) and in the SNr (n = 5 sham and n = 6 6-OHDA rats). At the end of each recording session all the animals were sacrificed and the brains used for histology and biochemical studies.

Open-field locomotor activity

Spontaneous horizontal motor activity, rearing (or vertical activity) and stereotypic movements were measured using a photoelectric actimeter (Actitrack, Panlab, S.L., Barcelona, Spain) (Belujon et al., 2007; Chetrit et al., 2009; Delaville et al., 2012). The apparatus consisted of a transparent cage that was connected to a photoelectric cell and locomotor activity was detected by light beams. All testing in the actimeter was done in an isolated room between 8:30 A.M. and 2:00 P.M. The protocol consisted of two phases: first, the animals were submitted to a habituation: spontaneous locomotor activity was recorded during three consecutive days over two sessions of 10 min each day. Then on day 4, the first 10-min session was used to establish the daily habituation and only the locomotor activity recorded during the second session of 10 min was used for data analysis. Values obtained on day 4 from sham and 6-OHDA rats were compared using the Mann–Whitney U test (Prism, GraphPad Software, San Diego, CA, USA).

Stepping test

The stepping test is designed as a useful test to monitor lesion-induced changes in forelimb akinesia in the rat that may be analogous to limb akinesia seen in PD (Olsson et al., 1995). Asymmetry in forelimb motor activity of GP unilateral DA-depleted rats was assessed by measuring the number of adjusting steps on a smooth-surfaced table. The rat was held by the experimenter with one hand softly blocking the hind limbs (slightly raising the torso and the hind limbs above the surface) and with the other hand fixing the forelimb not to be monitored. In this way the other forepaw had to bear the body weight. The rat was moved slowly sideways in both right and left directions by the experimenter with a speed of 0.9 cm per 5 s (Fang et al., 2006). This was done for both the contralateral and ipsilateral forepaws. When the rat was moved along the table, the free forelimb had to step with the movement of the experimenter to keep balance. The number of adjusting steps for both directions and both paws was counted. Values of the contralateral paw vs. ipsilateral paw were compared using the Mann–Whitney U test (Prism, GraphPad Software, San Diego, CA, USA).

Electrophysiological recordings

To perform electrophysiological recordings, rats were anesthetized with urethane (1.2 g/kg) and placed in a stereotactic frame (Unimecanique, Paris, France). Body temperature was measured rectally and maintained around 37 ± 0.5 °C throughout the experiment by use of a heating pad. Recordings of STN and SNr neurons were done in sham and 6-OHDA rats. As previously described (Bouali-Benazzouz et al., 2009; Chetrit et al., 2009), the skull and dura mater overlying the STN and SNr were carefully removed and single-unit recordings performed in the STN and SNr. A single glass micropipette electrode (WPi, Inc., Hertfordshire, UK) (impedance: 8–12 MΩ) filled with 1% Pontamine sky blue dissolved in 3 M NaCl was then inserted vertically into the right structure target according to the coordinates given in the brain atlas of Paxinos and Watson (1998) (AP, 3.4–4.0 mm posterior to bregma; L, 2.2 – 3.0 mm from the midline; D, 7.2–8.6 mm from the dura for the STN; AP, 5.2–5.8 mm posterior to bregma; L, 2.3–2.9 mm from the midline; D 7.4–9.2 mm from the dura for the SNr). Extracellular neuronal activity was amplified, bandpass-filtered (300–3000 Hz) using a preamplifier