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Research Paper

Differential effects of levodopa and apomorphine on neuronal population oscillations in the cortico-basal ganglia loop circuit in vivo in experimental parkinsonism

Johanna Kühn[a], Jens K. Haumesser[a], Maximilian H. Beck[a], Jennifer Altschüler[a], Andrea A. Kühn[a], Vadim V. Nikulin[a,b], Christoph van Riesen[a,*]

[a] Charité University Medicine Berlin, Department of Neurology, Berlin, Germany
[b] Max Planck Institute for Human Cognitive and Brain Sciences, Department of Neurology, Leipzig, Germany

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ABSTRACT

The current pharmacotherapy of Parkinson's disease (PD) is primarily based on two classes of drugs: dopamine precursors, namely levodopa, and dopamine receptor agonists, such as apomorphine. Although both types of agents exert their beneficial clinical effects on motor and non-motor symptoms in PD via dopamine receptors, clinical efficiency and side effects differ substantially between levodopa and apomorphine. Levodopa can provide a greater symptomatic relief than dopamine receptor agonists. However, because long-term levodopa use is associated with early debilitating motor fluctuations, dopamine receptor agonists are often recommended in younger patients. The pharmacodynamic basis of these profound differences is incompletely understood. It has been hypothesized that levodopa and dopamine receptor agonists may have diverging effects on beta and gamma oscillations that have been shown to be of importance for the pathophysiology of PD. Here, we used electrophysiological recordings in anesthetized dopamine-intact and dopamine-depleted rats to systemically compare the impact of levodopa or apomorphine on neuronal population oscillations in three nodes of the cortico-basal ganglia loop circuit. Our results showed that levodopa had a higher potency than apomorphine to suppress the abnormal beta oscillations often associated with bradykinesia while simultaneously enhancing the gamma oscillations often associated with increased movement. Our data suggests that the higher clinical efficacy of levodopa as well as some of its side effects, as e.g. dyskinesias may be based on its characteristic ability to modulate beta-/gamma-oscillation dynamics in the cortico-basal ganglia loop circuit.

1. Introduction

Parkinson's disease is a chronic neurodegenerative disorder that affects about 1% of the population over the age of 60 years (de Lau and Breteler, 2006). In the course of the disease a progressive loss of dopamine in the basal ganglia results in a debilitating movement disorder. The symptomatic pharmacologic treatment of Parkinson's disease is mainly based on dopaminergic drugs, i.e. levodopa and dopamine receptor agonists. Although both classes of medication are effective at reducing motor symptoms, evidence shows that levodopa provides a greater symptomatic relief than dopamine receptor agonists and that levodopa is associated with fewer side effects (Connolly and Lang, 2014). However, as long-term use of levodopa is thought to cause motor fluctuations such as dyskinesias, it is not recommended as an initial treatment for younger patients (Ferreira et al., 2013). Since both lines of dopaminergic treatments are accompanied by substantial drawbacks, there should be a further optimization of dopaminergic therapeutics. For the future development of dopaminergic drugs with a higher clinical efficiency it is imperative to better understand how levodopa and dopamine receptor agonists impact on activity in the neuronal networks of the basal ganglia and cortex.

Converging evidence from invasive recordings of human PD patients and from animal models of the disease has demonstrated that abnormal beta and gamma oscillatory activity in cortico-basal ganglia loop circuits are paramount for the pathophysiological understanding of the disease (Brown et al., 2001; Kuhn et al., 2005; Kuhn et al., 2004; Leblois et al., 2007; Levy et al., 2002; Sharott et al., 2005; Shimamoto et al., 2013). It is currently hypothesized that enhanced beta and suppressed gamma oscillatory activity are directly linked to clinical symptomatology. Effective pharmacologic treatments with levodopa as well as with dopamine receptor agonists reduce pronounced beta and enhance gamma activity in patients (Kuhn et al., 2009; Ray et al., 2008). Since

* Corresponding author at: Charité University Medicine Berlin, Movement Disorder and Neuromodulation Unit, Charitéplatz 1, 10117 Berlin, Germany.
E-mail address: christoph.van-riesen@charite.de (C. van Riesen).

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these changes in oscillatory basal ganglia activity correlate with the alleviation of the clinical symptoms, it has been argued that the clinical improvement by dopaminergic drugs is directly mediated via changes of oscillatory activity in the cortico-basal ganglia loop on a system level (Kuhn et al., 2006; Litvak et al., 2012; Sharott et al., 2014). However, it has not yet been systematically tested whether levodopa and dopamine receptor agonists differ in their ability to influence the neuronal population oscillations arising in multiple nodes of the cortico-basal ganglia loop circuits.

The primary aim of our study was to investigate the effectiveness of levodopa and the dopamine agonist apomorphine to modulate pathological beta and gamma oscillations in the cortico-basal ganglia loop in the 6-OHDA model of PD in order clarify two important open questions. First, we wanted to find the pharmacodynamic basis for the apparent differential clinical potency of these classes of drugs by measuring neuronal population activity. Secondly, we also aimed at improving our understanding of the role of beta and gamma oscillations in the complex pathophysiology of PD by studying the dynamic interactions of beta and gamma oscillations under varying levels of different dopaminergic medications.

To address these aims, we performed simultaneous electrophysiological recordings from the primary motor cortex, the subthalamic nucleus and the reticulate part of the substantia nigra in parkinsonian rats and dopamine-intact rats under three increasing doses of levodopa or the dopamine receptor agonist apomorphine.

2. Material and methods

2.1. Animals and materials

Animal procedures were performed on 45 adult male Wistar rats (Harlan Winkelmann, Germany) and were conducted in accordance with German Animal Welfare Act (last revised in 2014) and European regulations (2013/63/EU). All experiments were approved in advance by the local animal welfare authority (LaGeSo, Berlin) and conformed to local department and international guidelines. Group sizes and experiment-related discomfort were kept to the minimum possible. Animals were housed under standard conditions with a reversed 12 h light/dark cycle and free access to food and water. 24 rats received a unilateral dopamine (DA) denervating lesion as described below, 21 were kept as dopamine-intact controls. Unless stated otherwise, all chemical substances and drugs were obtained from Sigma Aldrich, Germany.

2.2. Unilateral 6-OHDA lesion model of PD

Rats were anesthetized with a combination of fentanyl (5 µg/kg, s.c., Rotexmedica, Germany), medetomidine (150 µg/kg, s.c., Provet AG, Germany) and midazolam (2 mg/kg, s.c., Hameln Pharma, Germany). After shaving the head rats were placed in a stereotaxic frame (David Kopf Instruments, CA, USA), heads fixed in horizontal cranium position with atraumatic ear bars. Corneal dehydration was prevented using ophthalmic ointment (dexpanthenol, Bepanthen ®, Bayer, Germany), body temperature was maintained at 37 ± 0.5 °C with a self-adjusting heating pad and respiratory rate was monitored to ensure the animals' wellbeing throughout the surgery. A midline scalp incision was made and the epicranial aponeurosis was removed to visualize the sutures. Bregma was used as reference point for stereotaxic targeting. All coordinates were measured in millimetre from bregma in the anterior-posterior (AP), medial-lateral (ML) and dorsal-ventral (DV) plane according to standard stereotaxic atlas (Paxinos, 2013).

A small craniotomy (Ø 1 mm) was made and a 33-gauge blunt cannula was inserted into the left medial forebrain bundle (MFB, AP: − 2.6, ML: + 1.6, DV: − 8.4). A volume of 1 µl of the neurotoxin 6-hydroxydopamine-hydrochloride (6-OHDA, dissolved in sodium chloride 0.9% containing 0.02% ascorbic acid at a final concentration of 8 µg/µl of the free base, stored at − 80 °C) was injected at a rate of 0.125 µl/min via a precision syringe pump system (10 µl Hamilton syringe and Micro4™ pump, World Precision Instruments, FL, USA). The cannula was left in place for 5 min after completion of the injection to prevent reflux of the liquid. After the removal of the syringe, the wound was sutured and anaesthesia was reversed with a combination of naloxone (120 µg/kg, s.c., B.·Braun Melsungen AG, Germany), flumazenile (200 µg/kg, s.c., Inresa, Germany) and atipamezole (750 µg/kg, s.c., cp-pharma, Germany). Carprofene (5 mg/kg, s.c., Pfizer, Germany) was given as analgesic on the first three postsurgical days. Behavioural tests and post-mortem immunochemistry (both see below) were performed to prove the success of the lesion.

2.3. Behavioural testing

Two well-established motor tests were performed, at baseline preceding the lesion and 20–35 days afterwards, immediately before the electrophysiological recordings. Dopamine-intact controls were assessed once before they underwent electrophysiological recordings. All behavioural experiments were documented via digital video recordings for subsequent offline analysis.

To assess motor behaviour, the cylinder test and the drag test were performed (Meredith and Kang, 2006). For the cylinder test animals were put in a transparent acrylic glass cylinder (height 45 cm, Ø 30 cm) without prior habituation and the use of the forepaws was quantified by counting full contacts with the wall during the animal's spontaneous vertical exploration (Schallert et al., 2000). A minimum of 15 touches within 10 min of at least one paw was required to complete the test. For the drag test the rats' lower body and hind limbs were raised while their forepaws remained at the ground. Then the rats were dragged backwards for the distance of one meter at a defined pace of 10 cm/s (Olsson et al., 1995). The use of the forepaws was assessed separately by counting the number of adjusting steps. A minimum of 15 steps of at least one paw was required to complete the test. Results of both tests are reported as a ratio of the right (affected) to the left (intact) side.

2.4. Electrode implantation and simultaneous electrophysiological recordings under urethane anaesthesia

Electrophysiological recordings were performed 20–35 days following the injection of 6-OHDA when a maximal lesion could be expected (Sharott et al., 2005). Surgical preparations correspond to those of the lesion apart from the following differences. Anaesthesia was induced and maintained with urethane (1.3 g/kg, i.p.). In total, 6 burr holes were drilled for electrode implantation. To record electrocorticograms (ECoG), two custom-made silver/silver-chloride electrodes (made out of 99.99% silver wire, Goodfellow, UK; spherical tip 0.5 mm, impedance 8 kΩ) were positioned epidurally above the left primary motor cortex, ipsilateral to the lesion (M1, AP: + 3.1/ + 2.9, ML: + 3.0; Fig. 1). Two silver/silver-chloride reference electrodes were placed in the epidural space above the cerebellum, one ipsilateral and one contralateral to the lesion (AP: − 8.0, ML: ± 3.0). Acrylic dental cement (Technovit®, Heraeus-Kulzer, Germany) and two short bone screws were used to fix all epidural electrodes. Next, two parylene insulated tungsten dual microelectrodes (Microprobes for Life Science, MD, USA; tip diameter 4 µm, tip separation 250 µm) were positioned above the target coordinates of the subthalamic nucleus (STN, AP: − 3.6, ML: + 2.5, DV: − 8.0) and the substantia nigra pars reticulata (SNr, AP: − 4.8, ML: + 2.5, DV: − 8.0; Fig. 1C). The animal was then placed in a faraday cage and final positioning of the depth electrodes was accomplished under recording and constant online evaluation of multi-unit activity (MUA) firing patterns along the trajectory as described previously (Beck et al., 2016). When both targets were reached, cortical electrodes were referenced against the ipsilateral cerebellum, basal ganglia electrodes against the contralateral side and baseline recordings were started. The wide band signals of the depth electrodes