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Dissociation of metabolic and hemodynamic levodopa responses in the 6-hydroxydopamine rat model

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ABSTRACT

Dissociation of vasomotor and metabolic responses to levodopa has been observed in human subjects with Parkinson's disease (PD) studied with PET and in autoradiograms from 6-hydroxydopamine (6-OHDA) rat. In both species, acute levodopa administration was associated with increases in basal ganglia cerebral blood flow (CBF) with concurrent reductions in cerebral metabolic rate (CMR) for glucose in the same brain regions. In this study, we used a novel dual-tracer microPET technique to measure CBF and CMR levodopa responses in the same animal. Rats with unilateral 6-OHDA or sham lesion underwent sequential ^{15}O-water ($H_2^{15}O$) and ^{18}F-fluorodeoxyglucose (FDG) microPET to map CBF and CMR following the injection of levodopa or saline. A subset of animals was separately scanned under ketamine/xylazine and isoflurane to compare the effects of these anesthetics. Regardless of anesthetic agent, 6-OHDA animals exhibited significant dissociation of vasomotor (ΔCBF) and metabolic (ΔCMR) responses to levodopa, with stereotyped increases in CBF and reductions in CMR in the basal ganglia ipsilateral to the dopamine lesion. No significant changes were seen in sham-lesioned animals.

These data faithfully recapitulate analogous dissociation effects observed previously in human PD subjects scanned sequentially during levodopa infusion. This approach may have utility in the assessment of new drugs targeting the exaggerated regional vasomotor responses seen in human PD and in experimental models of levodopa-induced dyskinesia.

1. Introduction

A number of early studies were conducted to examine the vasomotor effects of dopaminergic drugs in animal models and human subjects (Leenders et al., 1985; McCulloch and Edvinsson, 1980). Studies in different species showed that the action of these agents was mediated by dopamine receptors on cerebrovascular smooth muscle cells (Edvinsson et al., 1978; Toda, 1976) and on neighboring astrocytes (Ruscher et al., 2012). Dopamine binding at these sites regulates vasomotor tone in a reversible, dose-dependent manner and maintains local neurovascular coupling (Edvinsson et al., 1985; Filosa et al., 2016; Guell et al., 1982).

The regional cerebrovascular effects of levodopa are noteworthy. This molecule crosses the blood brain barrier (BBB) through endothelial cells on brain capillaries. Studies in 6-hydroxydopamine (6-OHDA) rat models have found that chronic treatment induces changes in these blood vessels potentially altering BBB permeability and transport kinetics (Lindgren et al., 2009; Ohlin et al., 2011; Ohlin et al., 2012; Westin et al., 2006). Of note, these microvascular changes were observed mainly in chronically levodopa-treated animals developing abnormal dyskinetic movements in response to drug. Indeed, abnormal central handling of exogenously administered levodopa is regarded as the critical upstream trigger for these movements (Cenci, 2014).

Dissociation of neuronal and vasomotor effects has been reported as a consistent feature of acute levodopa administration in human Parkinson's disease (PD). We used dual-tracer imaging with ^{15}O-water ($H_2^{15}O$) and ^{18}F-fluorodeoxyglucose (FDG) PET in human PD subjects to monitor the changes in regional cerebral blood flow (CBF) and cerebral metabolic rate for glucose (CMR) that occurred during acute levodopa administration (Hirano et al., 2008; Jourdain et al., 2015). In these individuals, intravenous levodopa infusion lowered local CMR in

Abbreviations: BBB, blood brain barrier; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; $H_2^{15}O$, ^{15}O-water; FDG, ^{18}F-fluorodeoxyglucose; CBF, cerebral blood flow; CMR, cerebral metabolic rate for glucose; GP, globus pallidus; LID, levodopa-induced dyskinesia; VOI, volume-of-interest; DI, dissociation index; EP, enteropeduncular nucleus; M2, auxiliary motor cortex.

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the putamen, globus pallidus (GP)/subthalamic nucleus, ventral thalamus, and dorsal pons – areas with abnormally elevated metabolic activity in the baseline unmedicated ("off") condition (Asanuma et al., 2006). By contrast, CBF measurements acquired concurrently in the same subjects during drug infusion revealed consistent dissociation of the vasomotor (ΔCBF) and metabolic (ΔCMR) levodopa responses, with increases, rather than reductions in the treatment ("on") condition (Hirano et al., 2008). While localized dissociation was not seen during comparably efficacious STN stimulation, these effects were found to be exaggerated in PD subjects with levodopa-induced dyskinesia (LID) (Hirano et al., 2008; Jourdain et al., 2015).

An autoradiographic study of levodopa-mediated changes in regional CBF and glucose utilization was conducted in different groups of 6-OHDA lesioned rats (Ohlin et al., 2012). Whereas large increases in regional CBF were seen in the basal ganglia following levodopa administration, corresponding changes in regional glucose utilization were modest or altogether absent. While the autoradiographic findings are compatible with earlier observations in the human, levodopa-mediated dissociation in the rodent cannot be fully evaluated with this approach. Indeed, to localize and quantify levodopa-mediated dissociation in the rodent model, concurrent CBF and CMR measurements are needed from single animals scanned both at baseline and following administration of drug. To this end, we modified the dual-tracer approach for use in small animals scanned with microPET before and after levodopa administration. Rats with unilateral 6-OHDA lesions were scanned with $H_2^{15}O$ and FDG microPET to map CBF and CMR at baseline and following the injection of levodopa or saline. Whole brain voxel-wise searches were conducted to identify regions with significant dissociation of vasomotor and metabolic responses to acute levodopa administration. In the first set of studies, scanning was performed under ketamine/xylazine anesthesia. Ketamine has been shown variably to affect regional CBF and CMR measurements in the rat brain (e.g., Cavazzuti et al., 1987). We therefore performed a set of microPET studies under isoflurane to determine whether levodopa-mediated dissociation effects were influenced by the choice of anesthetic agent.

2. Materials and methods

2.1. Subjects

A total of 41 female Sprague-Dawley rats (200–350 g; Harlan, The Netherlands) were housed with one littermate per cage under a 12 h light/dark cycle with access to food and water ad libitum. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) at The Feinstein Institute for Medical Research in Manhasset, NY and by the Malmo-Lund Ethical Committee on Animal Research in Lund, Sweden.

2.2. Treatment groups and experimental design

2.2.1. Ketamine/xylazine

Rats with a unilateral 6-OHDA lesion of the medial forebrain bundle (n = 20) or a sham lesion (n = 21) received a single subcutaneous injection of levodopa or saline (1.0 mL/kg body weight) 30 min prior to anesthetic induction with ketamine and scanning on the Siemens Inveon (Siemens AG, Munich, Germany) microPET system at The Feinstein Institute for Medical Research.

2.2.2. Isoflurane

A subgroup of the ketamine animals were restudied under isoflurane anesthesia in two additional scanning sessions, each conducted at least one week after the other. The isoflurane animals had either unilateral 6-OHDA (n = 8) or sham (n = 7) lesion. In the first session, a subcutaneous saline injection (1.0 mL/kg body weight) was administered 30 min prior to anesthesia and subsequent brain imaging. In the second session, the animals received subcutaneous levodopa/benserazide 30 min prior to anesthesia and subsequent imaging.

2.3. Drugs

L-3,4-Dihydroxyphenylalanine (levodopa) methyl ester and the peripheral decarboxylase inhibitor benserazide-HCl (Sigma-Aldrich, Stockholm, Sweden) were dissolved in saline and co-administered subcutaneously at the doses of 10/15 mg/kg. The injection volume was 1.0 mL/kg body weight.

2.4. Surgical procedure

2.4.1. Dopamine-denervating lesion and behavioral screening

Rats received a unilateral injection of 6-OHDA-HCl (Sigma-Aldrich, Stockholm, Sweden) into the right ascending dopamine fiber bundle (medial forebrain bundle). Rats were anesthetized with an intraperitoneal injection of a 20:1 mixture of fentanyl and medetomidine (Apoteksbolaget AB, Stockholm, Sweden) and placed in a stereotaxic frame. 6-OHDA was dissolved in a solution of 0.02% ascorbic acid/saline (3.5 μg/μL) and injected at the following two sites in accordance with standard protocol (i) 2.5 μL at anteroposterior (AP) = −4.4, lateral (L) = −1.2, dorsoventral (DV) = −7.8; toothbar = −2.4; (ii) 2.0 μL at AP = −4.0, L = −0.8, DV = −8.0 L; tooth bar = +3.4 (coordinates in mm relative to Bregma) (Ohlin et al., 2012). Sham lesions were performed by injecting saline at the same coordinates as the 6-OHDA procedures.

Two weeks following surgical lesion, rats were tested for amphetamine-induced rotation (2.5 mg/kg D-amphetamine intraperitoneally; 90 min recordings). Only animals exhibiting >5 net full turns per minute in the direction ipsilateral to the lesion were selected for the experiments (Winkler et al., 2002). The extent of the lesion was verified with tyrosine hydroxylase immunohistochemistry in all animals at the conclusion of the study. All lesioned animals exhibited <10% residual staining in the dopamine-denervated striatum and were thus included in the study.

2.5. MicroPET

2.5.1. Ketamine studies

In all 41 animals (20 6-OHDA, 21 Sham), anesthesia was induced with an intraperitoneal injection of ketamine (100 mg/kg)/xylazine (10 mg/kg) cocktail 15 min after receiving a subcutaneous injection of saline or levodopa. Once absence of reflexes was confirmed, the lateral tail vein was catheterized with a 24-gauge needle with attached polyethylene tubing for injection of $H_2^{15}O$. Two 25-gauge intraperitoneal butterfly catheter lines were secured with transpore tape: on the right intraperitoneal cavity for FDG injection, and on the left cavity for ketamine/xylazine bolus anesthesia maintenance. Anesthesia was maintained as needed (0.3 mL/kg). After lines were secured, the animal was placed on the scanner platform, with the head centered in the camera field of view; the animal position was maintained until completion of both scans. For CBF scans, 37–74 MBq of $H_2^{15}O$ tracer was injected into the lateral tail vein line and a 4-min emission scan was immediately acquired. A between-scan interval of 10 min was allowed for [^{15}O] decay prior to the start of FDG scanning for concurrent CMR measurement. To this end, 37–74 MBq of FDG was injected in the previously secured right intraperitoneal line; a 45-min uptake period was allowed, prior to the acquisition of a 10-min emission scan which was followed by a 4-min transmission scan. At conclusion of both scans, animals were allowed to recover on a clean cage until regaining the righting reflex.

2.5.2. Isoflurane studies

In 15 animals (8 6-OHDA, 7 Sham), anesthesia was induced with 2.5% isoflurane in 100% oxygen, via a breathing mask, 15 min after a subcutaneous saline or levodopa injection. Anesthesia delivery remained