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In vivo imaging of disturbed pre- and post-synaptic dopaminergic signaling *via* arachidonic acid in a rat model of Parkinson's disease

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Background: **Parkinson's disease involves loss of dopamine (DA)-producing neurons in the substantia nigra, associated with fewer pre-synaptic DA transporters (DATs) but more post-synaptic dopaminergic D_2 receptors in terminal areas of these neurons.**
Hypothesis: **Arachidonic acid (AA) signaling *via* post-synaptic D_2 receptors coupled to cytosolic phospholipase A_2 ($cPLA_2$) will be reduced in terminal areas ipsilateral to a chronic unilateral substantia nigra lesion in rats given D-amphetamine, which reverses the direction of the DAT, but will be increased in rats given quinpirole, a D_2-receptor agonist.**
Methods: **D-Amphetamine (5.0 mg/kg i.p.), quinpirole (1.0 mg/kg i.v.), or saline was administered to unanesthetized rats having a chronic unilateral lesion of the substantia nigra. AA incorporation coefficients, *k** (radioactivity/integrated plasma radioactivity), markers of AA signaling, were measured using quantitative autoradiography in 62 bilateral brain regions following intravenous [1-^{14}C]AA.**
Results: **In rats given saline (baseline), *k** was elevated in 13 regions in the lesioned compared with intact hemisphere. Quinpirole increased *k** in frontal cortical and basal ganglia regions bilaterally, more so in the lesioned than intact hemisphere. D-Amphetamine increased *k** bilaterally but less so in the lesioned hemisphere.**
Conclusions: **Increased baseline elevations of *k** and increased responsiveness to quinpirole in the lesioned hemisphere are consistent with their higher D_2-receptor and $cPLA_2$ activity levels, whereas reduced responsiveness to D-amphetamine is consistent with dropout of pre-synaptic elements containing the DAT. *In vivo* imaging of AA signaling using dopaminergic drugs can identify pre- and post-synaptic DA changes in animal models of Parkinson's disease.**
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Keywords: Arachidonic acid; PLA_2; D_2 receptors; D-Amphetamine; Parkinson's Disease; Quinpirole; Substantia nigra; Lesion

Introduction

Parkinson's Disease (PD) is a progressive neurodegenerative disorder that affects nigrostriatal dopamine (DA) projections, resulting in depletion of DA in the basal ganglia (Hornykiewicz, 1982). Degeneration of pre-synaptic nigrostriatal DA projections leads to decreased DA synthesis and storage within pre-synaptic striatal nerve terminals (Heiss and Hilker, 2004). PD has been modeled in rats by unilaterally injecting the selective monoaminergic toxin, 6-hydroxydopamine (6-OHDA), into the substantia nigra or medial forebrain bundle (Gerlach and Riederer, 1996; Ungerstedt, 1971). Behavioral deficits occur 1 week after 6-OHDA. After 4 weeks, the animal is considered a model of late-stage asymmetrical PD (Yuan et al., 2005).

Pre-synaptic DA neurons possess tyrosine hydroxylase, the rate-limiting enzyme for DA synthesis, and the high-affinity DA reuptake transporter (DAT). Reduced levels of DAT have been reported in PD patients and in 6-OHDA lesioned rats (Chalon et al., 1999; Ichise et al., 1999; Ribeiro et al., 2002; Zuch et al., 2000). In contrast, post-synaptic D_2-receptors are upregulated (Cadet and Zhu, 1992; Chalon et al., 1999; Ichise et al., 1999; Nikolaus et al., 2003), particularly in the posterior putamen (Antonini et al., 1995; Ichise et al., 1999). Post-synaptic D_1-receptors have been reported to be unchanged (Corvol et al., 2004; Shinotoh et al., 1993), increased (Corvol et al., 2004), or decreased (Turjanski et al., 1997) in the caudate-putamen of PD patients and in 6-OHDA lesioned rats.

Post-synaptic D_2-receptors can be coupled via a G-protein to Ca^{2+}-dependent cytosolic phospholipase A_2 ($cPLA_2$) (Clark et al., 1991; Nilsson et al., 1998; Vial and Piomelli, 1995), which also is localized on post-synaptic membranes and dendrites and which, upon activation, selectively releases AA from membrane phospholipid (Garcia and Kim, 1997; Nilsson et al., 1998; Ong et al., 1999; Pardue et al., 2003; Vial and Piomelli, 1995). D_1-receptors are not normally coupled to AA release (Bhattacharjee et al., 2005), but can become coupled following a chronic lesion of the substantia nigra in rodent brain (Cai et al., 2002; Hayakawa et al., 2001).

We have developed a method to image AA signaling via PLA_2 in unanesthetized rodents, in terms of a regional brain AA incor-

Abbreviations: PD, Parkinson disease; DA, dopamine; DAT, dopamine transporter; AA, arachidonic acid; PLA_2, phospholipase A_2; $cPLA_2$, cytosolic PLA_2; 6-OHDA, 6-hydroxydopamine; $rCMR_{glc}$, regional cerebral metabolic rate for glucose.

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poration coefficient k^* (brain radioactivity/integrated plasma radioactivity) that is measured with quantitative autoradiography following the intravenous injection of radiolabeled AA. k^* is unaffected by changes in cerebral blood flow that might be affected by drugs or stress, thus reflects only changes in brain AA metabolism. The method is presented in detail elsewhere (Bhattacharjee et al., 2005; Chang et al., 1997; DeGeorge et al., 1991; Hayakawa et al., 2001; Ohata et al., 1982; Rapoport, 2003; Robinson et al., 1992; Soncrant et al., 1988). With it, we showed in unlesioned unanesthetized rats that acute administration of quinpirole, a D_2-receptor agonist, increased k^* for AA in brain regions with high densities of D_2-receptors, and that the increases could be blocked by the preferential D_2-receptor antagonist, butaclamol (Bhattacharjee et al., 2005; Bristow et al., 1998; Hayakawa et al., 2001). We also reported that D-amphetamine, which increases synaptic DA by increasing pre-synaptic DA release and reducing DA reuptake by the DAT, increased k^* for AA in a dose-dependent manner in brain areas rich in D_2-receptors (Bhattacharjee et al., 2006). D-Amphetamine's effects could be prevented entirely by pre-administration of raclopride, a selective D_2-receptor antagonist (Bhattacharjee et al., 2006; Kohler et al., 1985), indicating that they were mediated specifically by D_2-receptors.

We thought it of interest in this paper to use our fatty acid method to examine the effects of D-amphetamine compared with quinpirole on AA signaling in unilaterally 6-OHDA lesioned rats. Based on evidence that ipsilateral regions in these animals have decreased DAT but increased D_2-receptor densities (see above), we predicted that the AA signal would be increased following quinpirole but might be reduced following D-amphetamine, depending on the extent of pre-synaptic loss and DAT reduction. We therefore quantified k^* for AA on the intact and lesioned sides of chronically left-sided 6-OHDA lesioned rats acutely administered saline 1 ml/kg i.p., quinpirole 1.0 mg/kg i.v., or D-amphetamine 5.0 mg/kg i.p. These doses were chosen on the basis of our prior studies in which drug-induced changes in k^* were measured following injection of [1-^{14}C]AA (Basselin et al., 2005; Bhattacharjee et al., 2005; Bhattacharjee et al., 2006; Hayakawa et al., 2001).

Materials and methods

Chemicals and drugs

[1-^{14}C]AA in ethanol (50 mCi/mmol, 99% pure) was purchased from Moravek Biochemicals (Brea, CA). (−)-Quinpirole (LY-171,555) was obtained from Research Biochemicals International (Natick, MA). D-Amphetamine sulfate, HEPES, fatty acid-free bovine serum albumin, paraformaldehyde, and propylene glycol were purchased from Sigma Chemicals (St. Louis, MO). Sodium pentobarbital was purchased from Richmond Veterinary Supply (Richmond, VA).

Animals

This study was approved by the National Institute of Child Health and Human Development Animal Care and Use Committee (Protocol 03-012). The experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication 86-23). We obtained male 6-OHDA lesioned Fischer-CDF rats from Charles River Laboratories (Wilmington, MA). Briefly, 8.5±0.5-week-old rats were anesthetized with 43 mg/kg ketamine and 8.77 mg/kg xylazine, and the left substantia nigra was lesioned by a 3-min infusion of 12 μg/4 μl 6-OHDA in 0.9% saline (w/v) at the following coordinates: AP=−1.5, ML=+1.8, and DV=−7.5 mm from the bregma (Hayakawa et al., 2001). Two weeks after lesioning, the rats were shipped to our controlled animal facility having constant temperature, humidity, and lighting cycle (6:00 AM–6:00 PM), and had *ad libitum* access to food pellets and water. To assess the efficacy of the lesion, rats were tested 9±1 days and 25±3 days later for their response to *S*-(+)-apomorphine HCl, 0.5 mg/kg i.p. (Hayakawa et al., 2001; Ungerstedt, 1971). Only rats that completed at least 100 contralateral rotations in 20 min during both testing sessions were studied.

Arterial and venous catheter placement

Prior to surgery, the 14±1 week-old rats (5.5±1 weeks after 6-OHDA lesion) weighed 270±25 g. Polyethylene catheters (PE 50) (Becton Dickinson, Sparks, MD) filled with heparin (50 IU) in 0.9% saline were surgically implanted into the right femoral artery and vein under halothane (1–2.5% v/v in O_2) anesthesia. The incision was infiltrated with 1% lidocaine hydrochloride and closed with clips. The rat was loosely bound in a fast-setting cast with its upper body free, and the cast was attached to a wooden block. The rat was allowed to recover from anesthesia in a sound-dampened, temperature-controlled box for 3–4 h, to remove any effect of anesthesia on brain metabolism (Kimes et al., 1985; Sokoloff et al., 1977). Arterial blood pressure, heart rate, and rectal temperature were measured prior to and 11 min after quinpirole, and 55 min after saline or D-amphetamine.

Radiolabeled arachidonic acid infusion

An unanesthetized rat was administered 1.0 ml/kg saline i.p. (n=11), 5.0 mg/kg D-amphetamine in saline i.p. (n=7), or 1.0 ml/kg quinpirole in saline i.v. (n=7). Forty-five minutes after saline or D-amphetamine or 1 min after quinpirole, the rat was infused intravenously with 170 μCi/kg [1-^{14}C]AA in 2 ml of 5 mM HEPES buffer, pH 7.4, containing 50 mg/ml fatty acid free bovine serum albumin. Infusion was performed for 5 min and at a rate of 400 μl/min, using a pump (Model 22, Harvard Apparatus, Natick, MA), and timed arterial aliquots (70–100 μl) were collected and centrifuged during and for 15 min following infusion. At 20 min following the start of infusion, the rat was killed with i.v. sodium pentobarbital (60 mg/kg) and its brain was removed, quickly frozen in 2-methyl butane at −40 °C, and then stored at −80 °C until sectioning.

Radiolabeled unesterified AA in plasma

Plasma was extracted from the arterial samples with 3 ml chloroform/methanol (2:1 v/v) and 1.5 ml 0.1 M KCl (Folch et al., 1957). Using a Liquid Scintillation Counter (Model 2200CA, Packard Instruments, Downers Grove, IL), 100 μl of the lower organic phase was counted to determine plasma radioactivity.

Autoradiography

Brains were sectioned in the coronal plane at −20 °C using a cryostat (Hacker Instruments, Fairfield, NJ). At 100 μm intervals, sets of 3 adjacent 20 μm slices were placed on 22×44 mm