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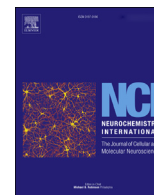

Reduced ability of calcitriol to promote augmented dopamine release in the lesioned striatum of aged rats

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A B S T R A C T

Parkinson's disease (PD) is a progressive and debilitating neurodegenerative disorder that affects over one million people in the United States. Previous studies, carried out in young adult rats, have shown that calcitriol, the active metabolite of vitamin D, can be neuroprotective in 6-hydroxydopamine (6-OHDA) models of PD. However, as PD usually affects older individuals, the ability of calcitriol to promote dopaminergic recovery was examined in lesioned young adult (4 month old), middle-aged (14 month old) and aged (22 month old) rats. Animals were given a single injection of 12 μg 6-OHDA into the right striatum. Four weeks later they were administered vehicle or calcitriol (1.0 μg/kg, s.c.) once a day for eight consecutive days. In vivo microdialysis experiments were carried out three weeks after the calcitriol or vehicle treatments to measure potassium and amphetamine evoked overflow of DA from both the left and right striata. In control animals treated with 6-OHDA and vehicle there were significant reductions in evoked overflow of DA on the lesioned side of the brain compared to the contralateral side. The calcitriol treatments significantly increased evoked overflow of DA from the lesioned striatum in both the young adult and middle-aged rats. However, the calcitriol treatments did not significantly augment DA overflow in the aged rats. Postmortem tissue levels of striatal DA were also increased in the young and middle-aged animals, but not in the aged animals. In the substantia nigra, the calcitriol treatments led to increased levels of DA in all three age groups. Thus, the effects of calcitriol were similar in the young adult and middle-aged animals, but in the aged animals the effects of calcitriol were diminished. These results suggest that calcitriol may help promote recovery of dopaminergic functioning in injured nigrostriatal neurons; however, the effectiveness of calcitriol may be reduced in aging.

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1. Introduction

Increasing evidence indicates that vitamin D has significant actions in the brain including regulation of calcium homeostasis (Gezen-Ak et al., 2011; Groves et al., 2014; Zanatta et al., 2012), modulation of neurotrophic factors (Neveu et al., 1994a; Sanchez et al., 2002; Saporito et al., 1994; Veenstra et al., 1997a) and neurotransmitter systems (Cass et al., 2012; Jiang et al., 2014; Sonnenberg et al., 1986), immunomodulation (Fernandes de Abreu et al., 2009; Kesby et al., 2011), and neuroprotection (Garcion et al., 2002; Groves et al., 2014; Kesby et al., 2011). The active metabolite of vitamin D_3 is calcitriol (1,25-dihydroxyvitamin D_3), which can cross the blood brain barrier to a limited extent (Gascon-Barre and Huet, 1983; Pardridge et al., 1985). In addition, the brain itself may be able to synthesize calcitriol (Eyles et al., 2005; Neveu et al., 1994b). The effects of calcitriol are mediated through genomic pathways, via the vitamin D receptor (VDR), and nongenomic pathways, via membrane bound VDR's or membrane-associated rapid response steroid binding receptors (Fernandes de Abreu et al., 2009; Garcion et al., 2002; Groves et al., 2014). Both types of receptors are found in the adult brain (Cui et al., 2013; Eyles et al., 2005, 2014; Pendyala et al., 2012; Prufer et al., 1999; Stumpf and O'Brien, 1987). Together these studies suggest that calcitriol has significant effects in the brain.

Parkinson's disease (PD) is a neurodegenerative disorder that is likely due in part to the progressive degeneration of nigrostriatal dopamine (DA) neurons. Several studies have linked deficiencies in vitamin D, or changes in the vitamin D receptor, with an increased risk of developing PD (Butler et al., 2011; Evatt et al., 2008; Newmark and Newmark, 2007). In animal models of PD, calcitriol has been shown to have beneficial effects. Using 6-

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hydroxydopamine (6-OHDA) models of PD, several studies have demonstrated that calcitriol can partially protect against the behavioral, neurochemical and histological effects of the toxin (Kim et al., 2006; Sanchez et al., 2009; Smith et al., 2006; Wang et al., 2001). Similarly, neuroprotective effects of calcitriol have been reported in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD (Kim et al., 2006). Two studies have also reported that calcitriol administration, starting 3–4 weeks after lesioning with 6-OHDA, was able to help restore tyrosine hydroxylase (TH) positive cells in the lesioned substantia nigra (Sanchez et al., 2009) and DA release and tissue levels in the lesioned nigrostriatal system (Cass et al., 2014). In addition, calcitriol has also been reported to be neuroprotective in several in vitro studies that have examined dopaminergic toxins or cells (for example see: Ibi et al., 2001; Jang et al., 2014, 2015b; Orme et al., 2013; Shinpo et al., 2000). Together, these studies demonstrate that calcitriol has neuroprotective and dopaminergic promoting properties in vitro, and in animal models of PD.

Although calcitriol has been shown to have beneficial effects in animal models of PD, previous studies have used young adult animals. PD is primarily a disease of the elderly, and there can be differences in how young and aged animals respond to pharmacological treatments. Thus, the present experiments were designed to examine and compare the ability of calcitriol to promote restoration of DA overflow and tissue content of DA in young adult (4 month old), middle-aged (14 month old) and aged (22 month old) rats previously lesioned with 6-OHDA. In vivo microdialysis was used to evaluate basal extracellular levels of DA and its primary metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and stimulus-evoked overflow of DA, from the striatum of rats treated with 6-OHDA and calcitriol. Postmortem tissue levels of DA in the striatum and substantia nigra were also evaluated to further asses the effects of calcitriol in the three age groups.

2. Materials and methods

2.1. Animals

Young adult (4 months old, 303–366 g), middle-aged (14 months old, 461–536 g) and aged (22 months old, 405–499 g) male Fischer-344 rats were obtained from Harlan Laboratories (Indianapolis, IN). The animals were housed in pairs under a 12-hr light-dark cycle with food and water freely available. All animal use procedures were approved by the Animal Care and Use Committee at the University of Kentucky and were in strict accordance with National Institutes of Health guidelines. Every effort was made to reduce the number of animals used and to minimize their pain and discomfort.

2.2. 6-OHDA injections

Animals were anesthetized with isoflurane (2.0–2.5% as needed) and positioned in a stereotaxic apparatus. Using sterile procedures, the skull was exposed, and a small hole drilled in the skull over the right striatum (1.0 mm anterior to bregma, 2.8 mm lateral from midline). A microliter syringe with a 26 gauge blunt tapered needle was used to inject 12 μg 6-OHDA (Sigma-Aldrich, St. Louis, MO) into the striatum 5.0 mm below the surface of the cortex. The 6-OHDA was injected in a total of 4 μl 0.9% saline with 0.1% ascorbic acid (pH 5.5) at a rate of 0.5 μl/min. The needle was left in place for an additional 5 min following the injection before being withdrawn. Gelfoam was placed in the burr hole, the incision closed with wound clips, and the animals allowed to recover.

2.3. Calcitriol treatment

Four weeks after the 6-OHDA injections the animals were injected once daily for eight consecutive days with calcitriol (1.0 μg/kg/day) or vehicle. All injections were administered subcutaneously. The calcitriol (Sigma Chemical Co., St. Louis, MO) was first dissolved in propylene glycol at a concentration of 100 μg/ml. For injections the calcitriol in propylene glycol was diluted into 0.9% saline so that the final volume given was 1 ml/kg of body weight. Vehicle treated animals were injected with propylene glycol diluted in 0.9% saline.

2.4. In vivo microdialysis

Microdialysis studies were conducted three weeks after the final calcitriol or vehicle treatment. The rats were anesthetized with urethane (1.25–1.50 g/kg, i.p.) and positioned in a stereotaxic frame. Body temperature was maintained at 37 °C with a heating pad coupled to a rectal thermometer. Microdialysis probes (CMA/11 probes, 3.0 mm length of dialysis membrane; CMA/Microdialysis, Acton, MA) were slowly lowered into both the left and right striata (0.0 mm anterior to bregma, 3.0 mm lateral from midline, tip of probe 6.3 mm below the surface of the brain). The probes were perfused at a rate of 1.2 μl/min with artificial cerebrospinal fluid containing 145 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.2 mM ascorbic acid, and 2.0 mM NaH_2PO_4 (pH 7.4). Dialysate fractions were collected at 20-min intervals. Following a 2-h equilibration period and the collection of 3 baseline fractions, DA overflow was stimulated by increasing the potassium concentration in the perfusate to 100 mM (NaCl reduced to 47.7 mM) for a single 20-min fraction, and then 2 h later by adding 100 μM D-amphetamine to the perfusate for a single 20-min fraction. Five final fractions with normal artificial cerebrospinal fluid were collected following the D-amphetamine stimulation. Dialysate samples were immediately frozen on dry ice and stored at −80 °C until assayed for DA, DOPAC and HVA.

2.5. Tissue collection and HPLC analysis

After collecting the dialysate fractions the urethane-anesthetized animals were killed by decapitation and their brains rapidly removed and chilled in ice-cold saline. A coronal slice of brain 2 mm thick at the level of the dialysis probes was made with the aid of an ice-chilled brain mold (Rodent Brain Matrix, ASI Instruments, Warren, MI). The location of all dialysis probes was confirmed to be centered in the dorsal striatum at the level of, or just rostral to, the crossing of the anterior commissure. The site of the intrastriatal injection was also visible and was confirmed to be located in the dorsal striatum. The striatum was then dissected from each half of the slice. The substantia nigra was dissected from both sides of a 2 mm thick coronal slice through the midbrain. The tissue pieces were placed in preweighed vials, weighed, and frozen on dry ice. Samples were stored at −80 °C until assayed for DA by high performance liquid chromatography (HPLC) as previously described (Cass et al., 2003). For dialysis samples, 20 μl of the dialysate was injected directly onto the column.

2.6. Data analysis

Dialysis probes were calibrated in vitro prior to use to determine acceptable probes (recovery of DA at least 12%). However, values were not corrected for in vitro recoveries as uncorrected values may be better correlated to true values (Glick et al., 1994). Basal levels of DA and metabolites were defined as the average value in the three fractions preceding stimulation by excess potassium.

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