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Effects of cinnarizine, a calcium antagonist that produces human parkinsonism, in parkin knock out mice

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Abstract

Cinnarizine, a calcium antagonist that produces parkinsonism in humans, induces behavioural changes such as alopecia, buco-lingual dyskinesia and reduction of motor activity in female parkin knock out (PK-KO) mice but not in wild-type (WT) controls. PK-KO mice have high striatal dopamine levels and increased dopamine metabolism in spite of low reduced tyrosine hydroxylase protein. Cinnarizine, which blocks dopamine receptors and increases dopamine release, further increased dopamine metabolism. PK-KO mice increased GSH levels as a compensatory mechanism against enhanced free radical production related to acceleration of dopamine turnover. Neuronal markers, such as β-tubulin slightly increased in PK-KO and furthermore with cinnarizine. Astroglial markers were decreased in PK-KO mice, and this effect was potentiated by cinnarizine, suggesting abnormal glia in these animals. Microglia was hyperactivated in PK-KO midbrain, suggesting inflammation in these animals. Proapoptotic proteins were increased by cinnarizine and, to a lesser extent, in PK-KO mice. Our data indicate that mutation of parkin is a risk factor for drug-induced parkinsonism.

Keywords: *Park-2* gene; Calcium antagonist; Dopamine neurons; Astrocytes; Microglia; Glutathione; Apoptosis; Locomotor activity; Dyskinesias; Parkinson's disease

1. Introduction

Drug-induced parkinsonism is frequent. It has been recognized for half a century as a common complication of antipsychotic drugs with a mean incidence in routine psychiatric practice from 10 to 15% of the patients (Tarsy, 1989). More recently, parkinsonism has been reported in patients taking other drugs such as antiemetics, antiarrhythmics, calcium channel antagonists, selective-serotonin reuptake inhibitors, lithium, valproic acid, procholinergics, chemotherapeutics, amphotericin B, estrogens, and others (Jimenez-Jimenez et al., 1997; Van Gerpen, 2002). Calcium channel

Abbreviations: DA, dopamine; DOPAC, 3, 4-dihydroxyphenyl-acetic acid; GSH, reduced glutathione; GSSG, oxidized glutathione; GSX, total glutathione; 5-HIAA, 5-hydroxy-indole-acetic acid; HVA, homovanillic acid; MAO, monoamino oxidase; MHPG, 4-hydroxy-3-methoxy-phenyl-glycol; 3-MT, 3-methoxy-tyramine; NA, noradrenaline; PD, Parkinson's disease; PK-KO, parkin knock-out; 5-HT, serotonin; TH, tyrosine hydroxylase; WT, wild type.

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antagonists induced parkinsonism is still a serious medical problem in some countries due to the wide use of these products in the elderly, in whom they produce irreversible deficits in up to one-third of the patients (Garcia-Ruiz et al., 1992b).

It has been debated whether drug-induced parkinsonism in general and progressive parkinsonism in patients receiving calcium channel antagonists, in particular, could take place in normal individuals or only in those with special genetic risk who would eventually develop this disease, such as patients with incidental Lewy body disease. In these patients or in individuals with subclinical deficits of the nigrostriatal dopamine system, the offending drug would not have a causative effect but would only play the role of unmasking a previously unrecognized akinetic syndrome. Cinnarizine, a calcium antagonist used in the treatment of headache and vertigo, produces persistent parkinsonism after short term oral administration to healthy monkeys (Garcia-Ruiz et al., 1992a), suggesting that some calcium channel antagonists could produce parkinsonism in healthy primates in a dose dependent pattern. The risk of drug-induced parkinsonism, however, is greater in individuals with a family history of parkinsonism or tremor than in individuals without affected relatives (Garcia-Ruiz et al., 1992b) suggesting that some genetic risk factor increases the risk in individuals exposed to these medications.

More than 10 genes or loci have been found to be associated with familial parkinsonism, transmitted according to Mendelian patterns of inheritance (Hardy et al., 2003). The most important of those genes so far discovered, in terms of frequency of mutations is *Park-2*, a gene that codes for a protein, parkin, with ubiquitin ligase function which also plays a role in dopamine (DA) release (Itier et al., 2003). Patients with homozygous or combined heterozygous mutations of *Park-2* develop early onset parkinsonism but individuals with single heterozygous mutations are usually asymptomatic. Knock out mice for parkin do not develop massive nigrostriatal degeneration but they present abnormal behaviour and DA neurotransmission. In order to investigate the relationship of calcium with the abnormalities of DA release found in parkin knock out mice, we have investigated the effect of cinnarizine, a calcium channel antagonist with powerful parkinsonising properties, in parkin knock out mice.

2. Materials and methods

2.1. Animals and treatment

Wild-type (WT) C57BL6/129SV and parkin-null mutant (PK-KO) littermates mice were obtained from Aventis Pharma SA laboratory (Vitry-sur-Seine, France) (Itier et al., 2003). Procedures using laboratory animals were in accordance with the European Union Directives. All efforts were made to minimize the number of animals used and their suffering.

Twenty-four, 14-month-old female mice were randomised in four experimental groups of six animals. Twelve WT animals were randomised into two groups of six animals treated with vehicle or cinnarizine. Twelve PK-KO mice were similarly randomised into two groups of PK-KO control or PK-KO + cinnarizine. Cinnarizine was administrated in the drinking water for six weeks, at a dose of 10 mg/kg/day. Food consumption and fluid intake were measured daily and the rate of body weight gain once a week. The amount of fluid containing cinnarizine taken by WT (175 ± 7.3 ml/kg/day) and PK-KO (169 ± 5.77 ml/kg/day) mice was similar.

2.2. Behavioural studies

Exploratory behaviour, social interaction and motor activity were measured in drug-naive animals and after pharmacological treatment with cinnarizine in the drinking water. Spontaneous motor activity was measured every week over a 30-min period, after 30 min of adaptation, using automated activity cages with photoelectric cells (Itier et al., 2003).

2.3. Brain regions and tissue preparation

After decapitation, brain parts were dissected, according to Carlsson and Lindqvist (1973) and the areas that contained a rich density of dopamine terminals, limbic system and striatum, or DA cell bodies (midbrain) were used for further analysis. The genotype of the animals was confirmed by Western blot analysis of the parkin protein in the brain using a parkin antibody. The brain parts were frozen on dry ice. The different brain regions were sonicated (VibraCell, level 2 for 30 s) in eight volumes (W/V) of 0.4 N perchloric acid with 0.5 mM $Na_2S_2O_5$ and 2% EDTA and then centrifuged at 12 000 rpm for 20 min at 4 °C. The supernatant was used for monoamines and their metabolites as well as for glutathione determination.

The pellet, with the proteins, was neutralized (W/V = 1/9) with the lysis buffer (0.75% Na_2CO_3, 2% SDS, 0.25 mM PMSF, 10 mg/ml leupeptin, 2 mg/ml aprotinin, 10 mg/ml pepsin) and then sonicated and centrifuged at 12 000 rpm for 30 min at 4 °C. The supernatant was used for protein determination by BCA assay and for electrophoresis analysis.

2.4. Western blot

Samples (20–50 μg) were added to SDS sample loading buffer 2× (10% glycerol, 2% SDS, 0.1%