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Research Report

Toxic influence of subchronic paraquat administration on dopaminergic neurons in rats

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ABSTRACT

Paraquat is a toxin suggested to contribute to pathogenesis of Parkinson's disease. The aim of the present study was to examine toxic influence of subchronic treatment with this pesticide (5 days, one injection per day, 2–3 days of withdrawal) on dopaminergic, serotonergic, noradrenergic and GABAergic neurons. Paraquat decreased the number of tyrosine hydroxylase-immunoreactive (TH-ir) neurons in the substantia nigra by 22% (measured 3 days after withdrawal). Two days after withdrawal the levels of the dopamine metabolites and dopamine turnover in the caudate–putamen, substantia nigra and prefrontal cortex were reduced by ca. 20–60%, and the binding of [^{3}H]GBR 12,935 to dopamine transporter dropped by 25–40% in the caudate–putamen. Three days after paraquat withdrawal, the level of dopamine in the caudate–putamen was significantly increased, and earlier decreases in DOPAC and HVA in the substantia nigra, as well as [^{3}H]GBR 12,935 binding in the caudate–putamen were reversed. Moreover, an increase in serotonin turnover in the caudate–putamen and prefrontal cortex, and noradrenaline level in the former structure was observed 2–3 days after paraquat withdrawal. Three days after the last paraquat injection 24–35% decreases in the proenkephalin mRNA levels and 5–7% reduction in glutamic acid decarboxylase (GAD)67 mRNA were found in the caudate–putamen. The present study suggests that subchronic paraquat administration triggers processes characteristic of early stages of dopaminergic neuron degeneration, and activates compensatory mechanisms involving dopaminergic, noradrenergic, serotonergic and GABAergic transmissions.

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Abbreviations: 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; DAT, dopamine transporter; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; HVA, homovanillic acid; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP$^+$, 1-methyl-4-phenylpyridinium; PENK, proenkephalin; TH, tyrosine hydroxylase; TH-ir, tyrosine hydroxylase immunoreactivity; VTA, ventral tegmental area

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1. Introduction

Parkinson's disease is a neurodegenerative disease whose main symptoms result from degeneration of dopaminergic neurons in the substantia nigra pars compacta, and a subsequent (80–90%) loss of dopamine in the striatum (putamen and caudate nucleus) (Ehringer and Hornykiewicz, 1960). Although the cause of this disease is unknown, so far, the contribution of some endo- or exogenous toxins which superimpose on individual genetic predisposition has been postulated (Di Monte et al., 2002).

Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) is a widely used herbicide which became a focus of neuroscientists' interest because of its close structural similarity to 1-methyl-4-phenylpyridinium (MPP^+) — a parkinsonism-inducing toxin (Davis et al., 1979; Langston et al., 1983). Since discovery of an association between the use of this pesticide in agriculture and incidence of Parkinson's disease, its contribution to pathophysiology of this disease has been postulated (Hertzman et al., 1990; Liou et al., 1997).

In spite of the fact that paraquat is a cation, it is able to pass blood–brain barrier after systemic administration in animals (Corasaniti and Nisticò, 1993; Shimizu et al., 2001; Barlow et al., 2003), *via* a specific neutral amino acid transporter (Shimizu et al., 2001). Its levels in the brain tissue are ca. 100 times lower than in the kidney (Barlow et al., 2003). When administered in *Rana temporaria*, paraquat markedly accumulated in the neuromelanin-containing, catecholaminergic neurons (Lindquist et al., 1988), which may suggest particular impendance of these neurons to its toxic effect. However, it is distributed relatively homogenously in rodent brains, probably because of the absence of the neuromelanin pigment in these species (Corasaniti and Nisticò, 1993; Barlow et al., 2003).

Data concerning the ability of this pesticide to trigger degeneration of dopaminergic neurons *in vivo* in rodents are equivocal. Brooks and coworkers (1999) have reported considerable losses of tyrosine hydroxylase-immunoreactive (TH-ir) neurons in the substantia nigra pars compacta (~60%) and their terminals in the striatum (~90%) of mice repeatedly treated with paraquat. Later studies, however, have reported no or only moderate decreases in the number of nigral neurons, striatal levels of dopamine and its metabolites, TH level and activity, or dopamine transporter (DAT) immunoreactivity (McCormack et al., 2002; Shimizu et al., 2003; Thiruchelvam et al., 2000a,b, 2003). Nevertheless, the toxic effect of paraquat on dopaminergic neurons has been found to be slightly increased by aging, α-synuclein mutation (A53T, A30P), co-administration with a fungicide — maneb or MPTP (Thiruchelvam et al., 2003, 2004; Shepherd et al., 2006).

Our recent studies indicate that a long-term (up to 6 months) paraquat administration (one injection per week) in rats induces a slowly developing degeneration of dopaminergic

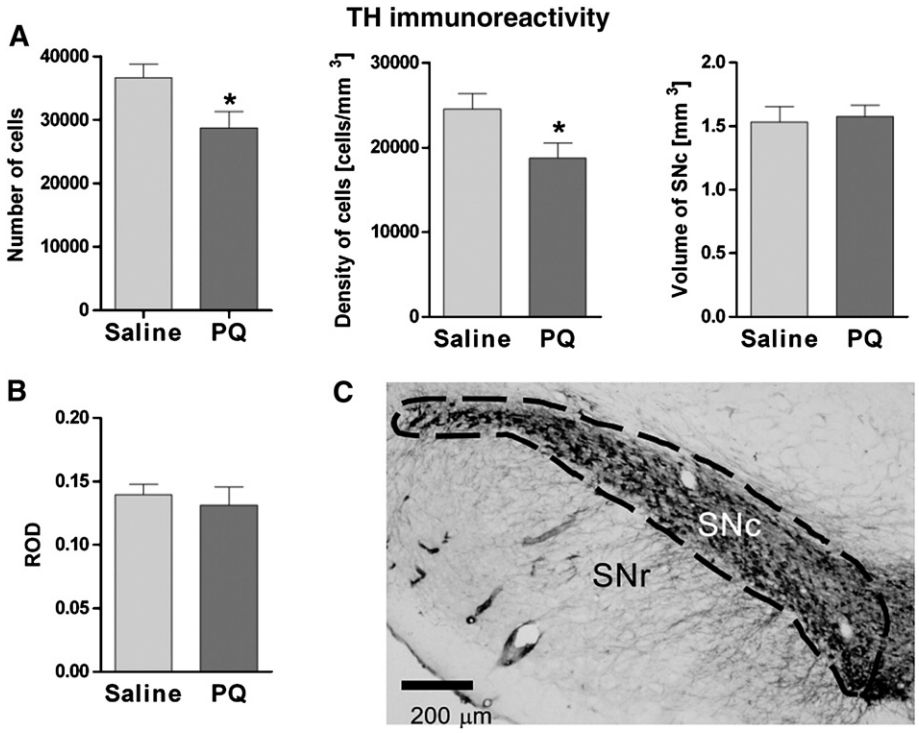

Fig. 1 – The influence of subchronic paraquat administration on: (A) the stereologically estimated number and density of tyrosine hydroxylase-immunoreactive (TH-ir) neurons and the volume of the substantia nigra pars compacta (SNc). (B) Relative optical density (ROD) of SNc immunostained for TH. The results are shown as the mean ± SEM. PQ — paraquat. $^*p < 0.05$ vs. control (saline-treated rats). Number of animals in groups: control: $n = 8$, PQ-treated animals: $n = 10$. (C) The analyzed region of SNc was outlined on an exemplary section immunostained for tyrosine hydroxylase. SNr — substantia nigra pars reticulata. Scale bar = 200 µm.