SHORT REPORT

Persistent loss of tyrosine hydroxylase immunoreactivity in the substantia nigra after neuroleptic withdrawal

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Abstract

A 37 year woman developed neuroleptic induced parkinsonism that persisted long after the drug had been discontinued. This prompted a study of the effect of an eight week course of haloperidol (HAL) followed by two week withdrawal, on dopaminergic neurons of the substantia nigra in rats. Animals treated with HAL showed a highly significant 32%–46% loss of tyrosine hydroxylase (TH) immunoreactive neurons in the substantia nigra, and 20% contraction of the TH stained dendritic arbour. Neuroleptic drug induced downregulation of nigral dopaminergic neurons may help to explain the persistent parkinsonism found in many patients after withdrawal of medication.

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Neuroleptic medications are among the most commonly used drugs for treating patients with serious mental illness. These agents, which for the most part act by blocking the D2 subtype of dopamine receptor, are associated with acute or subacute neurological side effects, which generally resolve when the medication is discontinued.^{1–4} These so-called “extrapyramidal” side effects, which include dystonia, parkinsonism, and akathisia, are thought to arise as a direct consequence of the dopamine receptor blockade in the striatum.

Neuroleptic drugs are also, however, associated with delayed onset, or tardive, syndromes, which typically begin after the patient has been taking the medication for some time, and which can persist for months, or even years, after the drug is discontinued.^{4–6} The existence of these tardive syndromes indicates that neuroleptic drugs are capable of producing long lasting changes in brain function. The nature of these long term changes, and the pathophysiology of the tardive syndromes, continue to be poorly understood.

Among the tardive syndromes which have been described are tardive dyskinesia,⁴ tardive dystonia,⁵ and tardive akathisia.⁶ There has been little attention to the potential of

neuroleptic drugs to produce tardive parkinsonism. We recently cared for a previously healthy young woman who had inappropriately received a five month course of high dose neuroleptic treatment, and who had persistent parkinsonism after the medication was withdrawn. This has prompted us to study the effects of neuroleptic medication on dopaminergic neurons of the substantia nigra.

Case report

A 37 year old married woman with no personal or family history of psychiatric or neurological illness consulted her family physician with complaints of anxiety, insomnia, sadness, inertia, and restlessness. Despite there being no evidence of psychosis, she was treated for five months with intramuscular injections of the neuroleptic fluphenazine decanoate (10–35 mg every two to four days), supplemented by oral fluphenazine (5–55 mg/day). On this regimen she became very slowed down, with a shuffling gait, mask-like face, cogwheel rigidity, and difficulty in writing. Because of her continuing parkinsonism the neuroleptic drugs were stopped, whereupon she developed pelvic rocking and gyrating. Six months later the patient was admitted to hospital. Examination at that time showed a flat affect, expressionless voice, and staring expression. There was cogwheel rigidity at the elbows and wrists, slowness and freezing during performance of alternate motion rate tasks, and micrographia. She maintained a stooped posture and had a slow shuffling gait with flexion at the elbows and no arm swing. The patient described an inner compulsion to move but displayed no spontaneous gesturing or movement, aside from constant pelvic rocking. Extensive laboratory investigations, including MRI, EEG, and evoked potentials, were all negative.

Over the ensuing five months the patient had trials of lorazepam, propranolol, and benzotropine, with minimal response. Treatment with levodopa/carbidopa and a course of electroconvulsive therapy given for her depression produced noticeable improvement in her parkinsonism, although she continued to have severe akathisia and pelvic dyskinesia. Eighteen months after her last dose of neuroleptic drugs there was still evidence of mild parkinsonism,

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Figure 1 TH immunoreactive cell counts (A) and cross sectional areas (B) in the substantia nigra of rats treated for eight weeks with saline (control), 1 mg/kg/day haloperidol (LOW HAL), or 10 mg/kg/day haloperidol (HIGH HAL), and then withdrawn from the treatment for two weeks. Both the number of TH positive neurons and the cross sectional area of TH immunoreactivity were significantly reduced in each of the haloperidol treated groups compared with controls. *** $p < 0.001$; **** $p < 0.0001$.

mild akathisia, and minimal pelvic dyskinesia. At a two and a half year follow up she had no evidence of parkinsonism, dyskinesia, or akathisia.

Materials and methods

EXPERIMENTAL STUDY

The study was carried out with male Sprague-Dawley rats, each weighing 250 g at the start of the experiment. They were housed in group cages, with free access to food and water, and maintained on a 12h:12h day:night light cycle. The animals were divided into three groups and received daily intraperitoneal injections of one of the following: (1) saline; CONT, $n=9$); (2) haloperidol (1 mg/kg/day; LOW HAL, $n=9$); (3) haloperidol (10 mg/kg/day; HIGH HAL, $n=9$). The daily injections were continued for eight weeks. Two weeks after the final injection the animals were overdosed with sodium pentobarbitone and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde. The brain was removed and postfixed overnight in 4% paraformaldehyde at 4°C. The tissue was then rinsed in 0.1 M phosphate buffer and cryoprotected in 15% sucrose solution. Frozen 40 μ m coronal sections of substantia nigra were cut on a Leitz cryostat and stored, six sections per well, at 4°C in 24 well plates containing 0.1 N phosphate buffered saline, pH 7.4.

Visualisation of dopaminergic neurons was achieved by tyrosine hydroxylase (TH) immunohistochemistry. Free floating sections containing substantia nigra were incubated at room temperature for 30 minutes in 0.3% hydrogen peroxide, then rinsed three times and placed in 5% normal goat serum for one hour. They were subsequently washed three more times, then incubated for 48 hours at 4°C with rabbit anti-TH antibody (Eugene Labs, NJ, USA) diluted 1:500. Further rinsing was followed by incubation for one hour with biotinylated second antibody (ABC Vectastain Elite Kit, Vector Labs, CA, USA). After another three rinses the sections were incubated in avidin-biotin solution, rinsed again ($\times 3$) then placed in a solution containing the chromogen diaminobenzidine tetrahydrochloride (DAB; Sigma, St Louis, MO, USA). The stained sections were mounted on gelatin coated glass slides, dried, dehydrated in ethanol (70%, then 95%, then 100%), plated in xylene, and cover-

slips added using Gurr DePeX mounting medium (BDH Labs, UK).

The mounted sections were analysed with the assistance of a MOP Videoplan video analysis unit (Kontron, Germany). The slides were coded and the TH immunoreactive cells in the substantia nigra were manually counted at $10\times$ magnification, without knowledge of the treatment group to which the slide belonged. The cross sectional area of the substantia nigra, encompassing all stained cells and their immunoreactive dendritic arbours, were mapped out at a magnification of $\times 2.5$, again with strict blinding to treatment status. Cell counts and cross sectional area of SN were determined on at least six sections from each animal, yielding mean values for each brain. The data were analysed statistically by one way analysis of variance (ANOVA), followed by post hoc t tests where appropriate.

Results

Figure 1 shows the counts of TH-immunoreactive cell bodies in the substantia nigra. There was a highly significant effect for group (ANOVA: $F(2,26)=22.18$; $p < 0.001$). The number of TH positive cells was reduced by 32% in the LOW HAL group compared with controls ($p < 0.001$) and by 44% in the HIGH HAL group ($p < 0.0001$). The difference between LOW HAL and HIGH HAL animals approached, but did not reach, significance ($p=0.06$).

Assessment of mean cross sectional area of the substantia nigra as outlined by TH immunohistochemistry also showed a significant effect for group ($F(2,26) = 24.83$; $p < 0.001$). Nigral area was reduced by 20% in the LOW HAL group ($p=0.001$) compared with controls and by 21% in the HIGH HAL group ($p < 0.0001$), with no difference between the two haloperidol treated groups.

Figure 2 shows a representative section from each of the three treatment groups.

Discussion

These results indicate that neuroleptic medications can produce a persistent down regulation of TH in dopaminergic neurons in the substantia nigra. Animals exposed to haloperidol for eight weeks and then withdrawn from the medication had a 32%-44% reduction in

Figure 2 Photomicrographs (2.5 $\times$ magnification) of TH positive immunoreactivity in the substantia nigra at the level of bregma -5.3 mm for representative sections from (A) saline treated controls; (B) LOW HAL animals; (C) the HIGH HAL group.

the number of TH immunoreactive nigral cell bodies, and a 20% decrease in nigral cross sectional area, two weeks after the drug had been discontinued.

To the best of our knowledge, this is the first study of TH immunohistochemistry in the substantia nigra after withdrawal of neuroleptic medications, in either animals or humans. Cottingham *et al*⁷ examined TH mRNA in the substantia nigra 16 hours after completion of a 19 day course of haloperidol (4 mg/day) and found no change. Weiss-Wunder and Chesselet⁸ studied animals treated with a four day course of fluphenazine mustard injections and reported reduced concentrations of TH mRNA in nigral neurons at two days and four days after completion of injections.

The ability of neuroleptic drugs to induce persisting suppression of TH in nigrostriatal neurons suggests that these agents may be capable of producing tardive parkinsonism. Melamed *et al*⁹ reported on two 35 year old patients with schizophrenia who, after many years of neuroleptic treatment, developed progressive parkinsonism that continued to worsen after discontinuation of the neuroleptic medication. Hardie and Lees¹⁰ found that 14 of 16 patients with neuroleptic drug induced parkinsonism

(median age 61) had incomplete resolution of the parkinsonism after prolonged withdrawal from neuroleptic drugs. In another series, five of 48 elderly patients with drug induced parkinsonism had persistent parkinsonian features seven weeks after stopping the drug, and another five initially improved but later went on to develop idiopathic parkinsonism.¹¹ The patient whom we studied, despite being young and despite having no previous neurological symptoms, had parkinsonism for at least 18 months after completing a five month course of high dose neuroleptic treatment.

The present results suggest that neuroleptic drugs might not only pose a risk of tardive parkinsonism: they might also predispose to the premature development of idiopathic parkinsonism. Neuroleptic medications are known to inhibit complex 1 of the electron transport chain.¹² Complex I deficiency has been reported in Parkinson's disease,^{13 14} and impairment of energy metabolism has been implicated in the pathogenesis of idiopathic parkinsonism.¹⁵ It is known that primate species, including humans, are far more sensitive than rodents to the neurotoxic effects of MPP⁺, which shares with neuroleptic drugs the ability to inhibit complex I. If a brief course of neuroleptic treatment, such as was used in the present experiment, can lead to persisting down regulation of a crucial enzyme in the biosynthesis of dopamine, longer term exposure to the medications, particularly in humans, might have more permanent effects.

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