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Dopamine turnover is upregulated in the caudate/putamen of asymptomatic MPTP-treated rhesus monkeys

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Abstract

In Parkinson's disease (PD) and experimental parkinsonism, losses of up to 60% and 80%, respectively, of dopaminergic neurons in substantia nigra, and dopamine (DA) in striatum remain asymptomatic. Several mechanisms have been suggested for this functional compensation, the DA-mediated being the most established one. Since this mechanism was recently challenged by striatal DA analysis in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys, we present data on several DAergic parameters in three groups of rhesus monkeys: MPTP-treated asymptomatic animals; symptomatic MPTP-treated animals with stable parkinsonism; and untreated sex and age matched controls. We determined ratios of striatal and nigral 3,4-dihydroxyphenyl acetic acid (DOPAC) to DA levels and tyrosine hydroxylase (TH) enzyme activity to DA levels, in addition to the commonly used homovanillic acid (HVA)/DA ratios which, as such, might be less reliable under the conditions of partial denervation. We found that in the asymptomatic MPTP monkeys the DOPAC/DA ratios in putamen and caudate nucleus were shifted with high statistical significance 1.9–5.8-fold, as compared to controls, the shifting of the ratios being in the same range as the 2.6–5.4-fold shifts in the symptomatic animals. Also TH/DA ratios were significantly increased in both, the asymptomatic and the symptomatic MPTP-treated monkeys, with shifts in the putamen and caudate nucleus of 3- and 2.7–7.0-fold, respectively. In the substantia nigra, DOPAC levels and TH activity were strongly decreased after MPTP (−77 to −97%), but the ratios DOPAC/DA and TH/DA were not changed in this brain region. Collectively, our findings support the concept of DAergic compensation of the progressive striatal DA loss in the presymptomatic stages of the parkinsonian disease process.

Keywords: Dopamine metabolites; Tyrosine hydroxylase; Dopamine turnover; Presymptomatic compensation; MPTP; Parkinson's disease

Parkinson' s disease (PD) is characterized by a progressive loss of the dopamine (DA)-containing pigmented perikarya in the substantia nigra and a marked loss of DA in the caudate nucleus and putamen (Agid et al., 1987; Hornykiewicz, 1998). The DA loss in the putamen is especially crucial, because the putamen is the input station of the basal ganglia motor circuits whose dysfunction results in the motor disorder of PD (Wichmann and DeLong, 1996). Although the severity of the motor symptoms roughly correlates with the degree of the striatal DA loss, about 80% DA loss is needed to produce clinically mild symptoms (Bernheimer et al., 1973); in animal models of PD, even higher degrees of striatal DA depletion are tolerated (Zigmond and Stricker, 1973). These observations have given rise to the notion that in PD there exists a preclinical stage during which the progressive DA loss is functionally compensated. In principle, functional compensation in the partially denervated striatum can be achieved by several, not necessarily mutually exclusive, mechanisms, such as: upregulation of DA turnover in the remaining neurons (Hornykiewicz, 1998; Hefti et al., 1985; Zigmond et al., 1984); ''passive stabilization'' of extrasynaptic DA levels (Bergstrom and Garris, 2003); extrastriatal DAergic mechanisms (Obeso et al., 2004); and/or through non-DAergic systems (Hornykiewicz, 1993; Bezard et al., 2003).

In PD, the ratio of striatal homovanillic acid (HVA)/DA as a measure of the synaptic DA turnover, was found to be already increased in patients with mild symptoms (Bernheimer et al., 1973): this suggested a compensatory upregulation in the remaining striatal DA neurons as a possible mechanism operative in the preclinical stages of the disease (Hornykiewicz, 1993). Increased striatal (DA metabolite)/DA ratios were also found in animal models of PD with various degrees of the nigrostriatal DA neuron loss (Zigmond et al., 1984; Hefti et al.,

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1980; Altar et al., 1987), compatible with the functional DAergic compensation concept.

In contrast to the conclusions from observations obtained in human PD and in a large number of animal, including primate, studies (Zigmond et al., 1984; Hefti et al., 1980; Altar et al., 1987; Elsworth et al., 1989; Di Paolo et al., 1986; Schneider, 1990; Sharman et al., 1967), stands the conclusion reached in a more recent study in monkeys treated with daily injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), thereby producing an acute and progressive parkinsonian state with presymptomatic and symptomatic striatal DA loss (Bezard et al., 2001). As a measure of striatal DA metabolism the authors used ratios of the combined DA metabolites (HVA + 3,4-dihydroxyphenyl acetic acid [DOPAC]) to DA. Because the observed apparent putamenal increases in these ratios were, in contrast to a clear and progressive striatal DA loss, statistically not significant (using one-way ANOVA followed by Bonferroni test, whereas a nonparametric test might have been more appropriate, see Fig. 3C in Bezard et al., 2001), the authors concluded that the presymptomatic compensation, including that in PD, does not involve DAergic mechanisms.

In a previous account based on a detailed regional brain study, we reported on the effect of MPTP on the levels of the three major brain monoamines DA, noradrenaline and serotonin in 45 brain regions of asymptomatic and symptomatic rhesus monkeys (Pifl et al., 1991). We now wish to communicate, for the first time, that part of our results dealing specifically with the metabolism of DA within the nigrostriatal system in this primate model of PD. We present, in addition to the DA levels, the levels of DOPAC, HVA, and the activity of tyrosine hydroxylase (TH) in the striatum and the nigra, so as to define, as fully as possible, the state of the nigrostriatal DA metabolism (in terms of ratios DOPAC/DA, HVA/DA, and TH/DA) in three well-defined groups of rhesus monkeys, i.e. (1) subjects in a permanent parkinsonian state; (2) asymptomatic monkeys that received a modified MPTP treatment regimen; and (3) age and sex matched controls.

The presented data could be expected to clarify the question of the role of the striatal DA in the phenomenon of presymptomatic compensation, being thus of utmost importance to our understanding of the mechanisms operative in the parkinsonian disorder.

1. Experimental procedures

MPTP–HCl (0.3% solution in distilled water) was administered i.m. to nine adult rhesus monkeys (four males and five females; weight: 4.3–12.2 kg; age: 10–22 years).

In a protracted treatment MPTP–HCl, in a total amount of 2.7–7.5 mg/kg, was given to five monkeys (three males, two females) in 9–23 single doses over a period of 2–7 months. The MPTP-free intervals were adjusted individually according to the response. Typically, treatment was started by two injections of 0.3 mg/kg MPTP–HCl separated by an interval of four drug-free days. Then doses of 0.3 mg/kg were administered not more frequently than once or twice per week. When signs of dopaminergic stimulation (yawning, sexual stimulation) appeared or the animal's general condition started to be impaired (e.g. reduction of food intake), the MPTP treatment was interrupted, to be resumed only after all symptoms had completely disappeared. In one monkey of the asymptomatic group, 32 daily doses of 0.1 mg/kg MPTP were administered (without any behavioural effects) within a period of 56 days.

In a more condensed administration MPTP–HCl, in a total dose of 2.1–6.45 mg/kg body weight, was administered to four monkeys (one male, three females) in 7–18 single doses over a period of 12–36 days. Treatment was started by injecting 0.15–0.3 mg/kg i.m. on each of three consecutive days. After a drug-free interval of 2 days, single injections (0.2–0.4 mg/kg), not exceeding one per day, were given intermittently until severe Parkinson-like symptoms had developed and remained stable for at least 1 week.

The degree of disability was assessed according to a disability score based on the symptom bradykinesia (Hinzen et al., 1986). The protracted treatment produced no parkinsonian signs and the five animals formed our asymptomatic group; by contrast the more condensed administration of MPTP resulted in a severe Parkinson-like condition over the period until sacrifice (2–10 weeks after the last MPTP dose; 6.5 months in one animal). This symptomatic group of four animals required the administration of therapeutically optimal daily doses of DA-substituting drugs, including L-DOPA and talipexole in order to minimize animal suffering and reduce the number of animals used; however, in the last 11–19 days before sacrifice, only direct acting DA agonists, but not L-DOPA, were administered. Seven monkeys acted as controls (four males and three females; weight: 5.9–14.4 kg; age: 7–23 years). The study was carried out under the supervision of the Animal Protection Office, Veterinary Administration of the District Authority of Rhein-Hessen-Pfalz. The monkeys were killed in deep anaesthesia with pentobarbital, the brain was removed from the skull and frozen at −80 °C until chemical analysis. Caudate nucleus and putamen were taken from rostral (precommissural) slices. Brain regions were dissected from frozen frontal slices of approximately 1–2 mm thickness. DA, DOPAC and HVA were determined by means of high-performance liquid chromatography (HPLC) with electrochemical detection (ED) as described previously (Pifl et al., 1991). TH activity was estimated by measuring the amounts of L-3,4-dihydroxyphenylalanine (L-DOPA) formed in homogenates from added L-tyrosine as described previously (Pifl et al., 1990). Samples were ultrasonicated in deoxygenated and nitrogen saturated ice-cold water and sodium phosphate buffer pH 6.0 and Triton-X 100 were added to a final concentration of 10 mM and 0.2%, respectively. Aliquots were incubated at 37 °C for 30 min in a shaking water bath in 0.1 M sodium acetate buffer, pH 6.0, 0.2 mM L-tyrosine, 20 mM 1-mercaptoethanol, 0.1 mM ferrous sulfate, 2.5 mM 2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine and 185 μg of catalase in a final volume of 200 μl. Reaction was started by addition of L-tyrosine and stopped by addition of 200 μl 0.2 M perchloric acid with 0.8 mM sodium bisulfite. After centrifugation and extraction with aluminium oxide L-DOPA was determined by HPLC/ED (Pifl et al., 1990).

Statistical significance between the three groups was calculated by a nonparametric test (Kruskal–Wallis *H*-test) due to the strongly differing variances in the three groups. Differences between asymptomatic and controls, symptomatic and controls and asymptomatic and symptomatic group were calculated by Mann–Whitney *U*-test.

2. Results

As found previously (Pifl et al., 1991), DA levels in the striatum and the substantia nigra were greatly decreased in both our MPTP-treated groups as compared with the control group ($p < 0.01$ by Kruskal–Wallis *H*-test, Table 1). In the asymptomatic monkeys DA in putamen and caudate nucleus was reduced by −92.1 and −94.5%, respectively. These levels, although low, were still 10–20-fold higher than in the striatum of the symptomatic animals ($p < 0.05$ by Mann–Whitney *U*-test), in whom DA levels were reduced by −99.3% in the putamen and −99.8% in the caudate nucleus. The substantia nigra DA was distinctly less affected by the MPTP treatment than the striatal DA. This was especially true for the asymptomatic subjects in whom the nigral DA levels were decreased by −68.7% (Table 1). In the symptomatic animals,