LIPID PEROXIDATION IN THE CAUDATE NUCLEI IN EXPERIMENTAL PARKINSONIAN SYNDROME

V. G. Kucheryants, M. A. Atadzhanov, E. V. Nikushkin, V. A. Zagorevskii, and L. M. Sharkova

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If dopamine is deficient in the caudate nuclei (CN) their cholinergic neurons undergo disinhibition and hyperactivation. As a result, a generator of pathologically enhanced excitation (GPEE) is formed in CN (2), leading to the development of a parkinsonian syndrome (1). To reproduce a parkinsonian syndrome, the substance 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its oxidative end product 1-methyl-4-phenylpyridinium (MPP^+), which possess selective toxicity toward nigrostriatal dopaminergic neurons [7, 9], is most widely used at the present time.

Since an important step in the pathogenesis of GPEE is disturbance of regulation of lipid peroxidation (LPO) in the central nervous system (CNS) [4], it was decided to study the state of LPO in CN in rats in which the model of a parkinsonian syndrome was created with the aid of MPTP and MPP^+.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats aged 9-10 months, weighing 400-550 g. A model of a parkinsonian syndrome was created by three methods: 1) intraperitoneal injection of MPTP hydrochloride into the animals (four injections, each of 20 kg/kg, with intervals of 12 h); 2) injection of MPP^+ in a dose of 20 μg; 3) injection of MPP^+ in a dose of 20 μg into both nuclei of the substantia nigra (SN) [3]. The coordinates of the points of injection of MPP^+ into CN were: A) 1, L) 2.5, H) 4.5: the points of injection of MPP^+ into SN were: A) 4, L) 2.0, H) 8.0 [11]. MPTP and MPP^+ were injected in physiological saline in volumes of 1 ml and 2 μl, respectively. Animals of the control group received injections of the corresponding volume of physiological saline.

The animals were kept under standard animal house conditions on an ordinary diet, and remained under observation for 24-120 h.

The motor activity of the animals was studied by the open field method, by recording the number of squares crossed by the animals in the course of 5 min (NSC). Muscular rigidity was assessed by the "lordosis" sign. Tremor was evaluated by means of tremograph, reccording the frequency and amplitude of shakings of the head, forelimbs, trunk and tail of the animal. All three signs – oligokinesia, rigidity, and tremor were evaluated on a point system [3]. The rats were decapitated 48 h after injection of MPTP and 24, 48, 72, and 120 h after injection of MPP^+.

The state of LPO was assesed by the level of products reacting with 2-thiobarbituric acid (TBA) in homogenates of the corpus striatum. Homogenates of the striatum were prepared in a ratio of 1:9 in medium containing (in mM): NaCl 150, EDTA 3, tris-HCl 10; pH 7.4 (20°C). To 100 μl in homogenate were added 1.5 ml of TCA (25-30%) and 1.5 ml of TBA (0.5%), after which the mixture was incubated at 50°C for 120 min. The optical density of the solutions was measured at the peak of absorption of the TBA products at 535 nm on a "Hitachi 320" spectrophotometer (Japan). Concentrations of TBA products were expressed in

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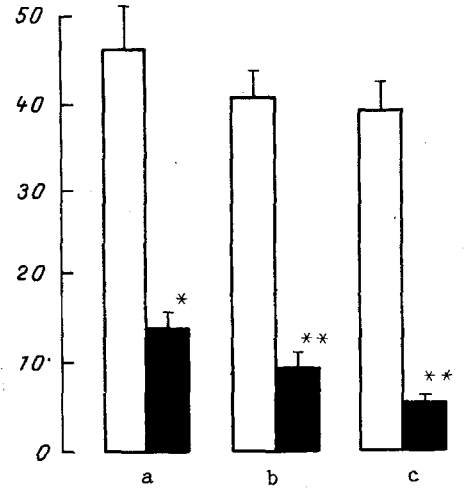

Fig. 1

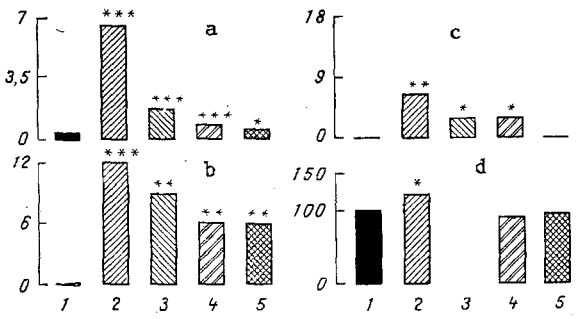

Fig. 2

Fig. 1. Changes in motor activity of rats after administration of MPTP and MPP^+ a) MPTP; b) MPP^+ into CN; c) MPP^+ into SN. Unshaded columns – control, black columns – neurotoxin. Vertical scale represents NSC. *p < 0.01, **p < 0.05 compared with control.

Fig. 2. Changes in severity of manifestations of parkinsonian syndrome and level of TBA products in striatum of rats after intranigral injection of MPP^+. a) Oligokinesia (relative units based on MSC); b) muscular rigidity (in points); c) tremor (in points); d) level of TBA products (in % of control). 1) Control; 2-5) 24, 48, 72, and 120 h, respectively, after injection of MPP^+.

nanomoles per milligram protein or per milligram phospholipid. The protein concentration was determined by Lowry's method. Phospholipids were determined by a set of standard reagents from "Boehringer" (Austria). The following reagents were used: MPTP and MPP^+ were synthesized at the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR; EDTA, 2-TBA, and TCA were from "Serva" (West Germany); Tris-HCl was from "Sigma" (USA); other reagents were of the chemically pure grade (USSR).

EXPERIMENTAL RESULTS

Systemic administration of MPTP and intracaudate and intranigral injection of MPP^+ induced all the basic motor signs of parkinsonism (oligokinesia, rigidity, and tremor) in the rats. Tremor appeared in the animals at the onset of the syndrome and was of short duration. Motor disturbances characteristic of the parkinsonian syndrome were most marked in rats after intranigral injection of MPP^+ (Fig. 1). A study of the time course of development of the characteristic features of the experimental parkinsonian syndrome showed that after injection of MPP^+ into SN the basic symptoms (ligokinesia, rigidity, and tremor) reached their maximal severity 24 h after injection of the neurotoxin. Later the severity of the symptoms gradually declined (Fig. 2).

Development of the parkinsonian syndrome after administration of MPTP was not accompanied by changes in the level of TBA products in the striatum (Table 1). During development of the parkinsonian syndrome induced by injection of MPP^+ into CN, a tendency was observed for the level of LPO products in the striatum to increase. Finally, the development of a parkinsonian syndrome as a result of injection of MPP^+ into SN was accompanied by a significant increase in the concentration of TBA products in the striatum (Table 1). This effect, incidentally, appeared when the concentration of TBA products was calculated per milligram of phospholipid and it was absent when the level of TBA products was expressed per milligram protein.

The results indicate that changes in concentration of LPO products in the rat corpus striatum (i.e., changes in the intensity of LPO) correlate with the presence and severity of a parkinsonian syndrome in the animals. For instance, characteristic symptoms of maximal severity were observed 24 h after injection of MPP^+ into SN. The greatest changes (elevation) in the level of TBA products also were observed in ths method of modeling the syndrome, and also 24 h after injection of MPP^+ (Fig. 2).

TABLE 1. Level of TBA Products and Phospholipids in Striatum of Rats Receiving MPTP and MPP (M ± m)

Experimental conditions	Control		Phospholipids, μg/mg tissue
	%/mg protein	%/mg phospholipid	
Control	100±13,6	100±10,1	54,0±3,4
MPTP, 48 h after	106,3±18,5	138,0±13,8	72,0±8,4
Control	100±54,7	100±9,1	68,0±4,4
MPP^+ into CN, 24 h later	138,5±7,8	132±20,6*	54,0±2,6
Control	100±2,8	100±4,3	48,0±2,4
MPP^+ into SN, 24 h later	102,0±2,8	115,0±5,2**	42,0±3,8

Legend. Concentration of TBA products in control taken as 100%. p* < 0.1, **p < 0.05.

The fact that MPP^+ was more effective than MPTP can evidently be explained on the grounds that MPP^+ is a terminal metabolite of MPTP in the brain [6], and gives a neurotoxic effect [7, 12].

The mechanisms of the principal morphological changes in the CNS in Parkinson's disease (degeneration of melanin-containing dopaminergic neurons in SN, the locus coeruleus, and other structures) is not yet known. It has been suggested that these disturbances are due to excessive accumulation of 6-hydroxydopamine (6-HDA) in these regions [5]. It is known, for instance, that intraventricular injection of 6-HDA into dogs causes the appearance of characteristic symptoms of Parkinson's disease in the animals [13]. The cytotoxic action of 6-HDA is evidently connected with its ability to form H_2O_2 by auto-oxidation in the neurons [5]. H_2O_2 accumulation is facilitated by the decrease in catalase and peroxidase activity in the brain of persons with Parkinson's disease [5]. It has been suggested that the presence of H_2O_2 activates LPO in nerve cells, leading to their degeneration [5].

Support for the free-radical hypothesis of damage to dopaminergic neurons in Parkinson's disease is also given by data obtained by Liorsen et al. [10]. These workers found a decrease in the selenium concentration in SN of these patients and also a twofold increase in the iron concentration in the glial and nerve fibers of patients with parkinsonism [14]. Meanwhile direct investigations of levels of LPO products in the CNS in Parkinson's disease have given contradictory results. On the one hand, elevation of the level of TBA products has been found in SN of patients (post mortem) [8], but on the other hand, reproduction of an experimental parkinsonian syndrome in mice was not accompanied by changes in the level of diene conjugates in the striatum [7].

The results of the present investigation confirm the importance of LPO in the mechanism of origin of the parkinsonian syndrome. Since activation of LPO was recorded in CN (i.e., in the structure in which GPEE are formed, and, moreover, at a time when GPEE activity is manifested most strongly), the results are fresh confirmation that disturbance of LPO regulation is an essential stage in the pathogenesis of GPEE. This is in good agreement with the results of our previous investigations [4].

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ACTH-DEPENDENT CORTICOSTERONE SECRETION INHIBITED BY THE ANTIOXIDANT INDOL

F. Z. Meerson, V. V. Malyshev,
V. A. Petrova, and E. B. Manukhina

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It was shown previously that preliminary administration of the antioxidant ionol (dibunol) significantly limited the rise of the blood cholesterol level in animals with emotional-painful stress [4]. However, the mechanism of this stress-limiting effect is not yet clear. In particular, it is not known whether the antioxidant influences so important a stage of the stress reaction as the ACTH-dependent response of corticosterone formation and secretion by the adrenals.

The aim of this investigation was to study this problem by assessing the effect of ionol on the response of the adrenals to triple injections of ACTH and also to emotional-painful stress.

EXPERIMENTAL METHOD

Experiments (five series) were carried out on male Wistar rats weighing 200-220 g. ACTH (corticotrophin, from Kaunas Endocrin Preparations Factory) was injected subcutaneously in a dose of 2 units/100 g body weight 3 times in the course of 6 h, at intervals of 2 h. This order of administration of ACTH approximately simulated 6-hourly exposure to stress. Stress was produced in the form of an anxiety neurosis by the method of Desiderato and co-workers [6].

Ionol was injected intraperitoneally in a dose of 60 mg/kg before the first injection of ACTH or before the beginning of exposure to emotional-painful stress, daily for 4 days. The plasma and adrenal corticosterone levels were determined by the method of Botvin'ev [1]; extraction with methylene chloride was followed by chromatography on columns with silica-gel [3].

EXPERIMENTAL RESULTS

The results are evidence that the plasma corticosterone level in the control was 5.7 μg%, which corresponds according to data in the literature to the state of physiological rest [7]. The corticosterone concentration in the adrenals 1 h after the last of the three injections of ACTH was doubled, whereas its concentration in the blood plasma was trebled. Preliminary injection of ionol significantly (almost by half) reduced the increase in corticosterone concentration in the adrenals, and at the same time, almost completely prevented the rise of its concentration in the blood plasma.

Similar results were obtained during stress: 2 h after the end of exposure to stress the corticosterone concentration in the adrenals was doubled, and in the blood plasma it was trebled compared with the control. Preliminary administration of ionol led the increase

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