

PHARMACOLOGICAL CORRECTION OF CNS FUNCTIONAL DISORDERS AND PARKINSONIAN SYNDROME IN OLD ANIMALS

I.A. VORONINA, L.N. NEROBKOVA, O.A. KUTEPOVA and D.A. GUGUTCIDSE

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow

Summary. - The effects of 2-ethyl-6-methyl-3-hydroxypyridine (3-HP) on age-related and alcohol-induced impairment of memory and learning were studied in rats and mice. 3-HP was found to accelerate the acquisition of the conditioned reflex of active avoidance and to improve the retention of the conditioned reflex of passive avoidance in old (24 months) rats. 3-HP consumption during chronic (5 months) alcoholisation improved learning ability and prevented lipofuscin accumulation in brain of ethanol-treated mice. Extrapyramidal disorders after systemic administration of MPTP and intranigral injection of MPP⁺ depended on age of animals, dose of MPTP and MPP⁺, and duration of administration. The beneficial effects of 3-HP on age-related impairment of memory and learning and experimental parkinsonian syndrome may be due to its ability to inhibit the peroxidation of membrane lipids and increase cell resistance to different disturbing actions.

Riassunto (Interventi farmacologici su disordini funzionali del sistema nervoso centrale e sulla sindrome parkinsoniana in animali vecchi). - Sono stati valutati gli effetti della 2-etil-6-metil-3-idrossipiridina (3-HP) sulla perdita di memoria e della capacità di apprendimento dovuta all'invecchiamento o indotta dall'alcool in ratti e topi. Si è visto che la 3-HP favorisce l'apprendimento nei test di evitamento attivo e aumenta la memoria nei test di evitamento passivo nei ratti vecchi (24 mesi). La somministrazione di 3-HP aumenta le capacità di apprendimento e previene l'accumulo di lipofuscine nel cervello di topi trattati simultaneamente con etanolo per 5 mesi. Gli scompensi di tipo extrapiramidale indotti da MPTP somministrato s.c. e da MPP⁺ iniettato i.c. nella substantia nigra sono dipendenti dall'età degli animali (ratti), dalle dosi usate e dalla durata del trattamento. Gli effetti della 3-HP sui disturbi di memoria e di apprendimento correlati all'età e sulla sindrome parkinsoniana indotta

possono essere dovuti alla sua capacità di inibire la perossidazione dei lipidi di membrana e l'aumento della resistenza cellulare.

Introduction

The prominent impairments among age-dependent function disorders are reduction of CNS plasticity, decline of psychic adaptation of new and extreme environmental conditions, impairment of memory, intellect, motivation and sleep. According to the free radicals hypothesis of aging [1-3] most of the functional disorders occurring with age may be considered as free radicals pathology.

In this respect 3-hydroxypyridine derivatives that scavenge free radicals, change membrane physical and chemical properties, inhibit the peroxidation of membrane lipids [4, 5] should be regarded as possible geropsychotropic drugs.

Besides, one of the most common disease of brain aging is Parkinson's disease. Recently the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), its metabolites and ions are used in producing experimentally adequate model of parkinsonian syndrome (PS). In the monkey MPTP exhibits anatomical selectivity killing the dopaminergic (DA) cells in the *substantia nigra*, as well as a long lasting disappearance of central dopamine uptake sites [6, 7]. The peculiarity and mechanism of action of MPTP in rats is not yet clear. MPTP (2.5 and 10 mg/kg) was reported not to affect DA cells in rats [8-10]. However, these studies were carried out using low doses of MPTP, while biochemical and behavioural alterations were observed following administration of high doses of the drug.

It was shown that the concentration of dopamine in the striatal region of rats treated with three injections of MPTP (25, 30 or 50 mg/kg subcutaneously)

were reduced in a dose-dependent way. The maximal depletion was 43% and 58% of control [11]. The depletion of striatal dopamine in rat brain was observed after intranigral infusion of different doses of MPTP or its metabolite 1-methyl-4-phenyl, 1,2,3,6-tetrahydropyridinium (MPP⁺) [12-14] and after administration of MPTP in doses of 10 or 30 mg/kg subcutaneously [15, 16]. By means of histochemical methods it has been shown that intranigral injection of MPTP and MPP⁺ into the rat cause DA cell loss in the *substantia nigra* [17,18].

The first aim of the present study was to evaluate the effects of an antioxidant drug on the age-dependent impairments of memory and learning; the second direction of this study was to explore the possibilities of creation of an experimental PS in rats after high dose of MPTP and MPP⁺.

Materials and methods

The experiments were carried out on 3,6,15 and 24-month-old male albino rats and 3-month-old female mice ("Stolbovay" animal farm, Moscow region). The drug tested was 2-ethyl-6-methyl-3-hydroxypyridine (3-HP).

The influence of 3-HP on memory retention of the conditioned reflex of passive avoidance (CRPA) was tested on old (24 months) albino male rats. The passive avoidance test has been described elsewhere [19, 20]. The rats consumed 0.15% solution of 3-HP (40-75 mg/kg daily) instead of water for 7 weeks before, and 2 weeks after learning CRPA.

The conditioned reflex of active avoidance (CRAA) was formed in old (24 months) rats by means of shuttle-box techniques (Ugo Basile, Italy) as described elsewhere [20]. The rats consumed 0.15% solution of 3-HP (40-75 mg/kg/day) for two months before, and one week during the CRAA learning procedure.

Learning ability in alcohol-treated mice was estimated using the shuttle-box technique as reported elsewhere [21]. Three experimental groups of 3-month-old female mice were maintained on laboratory chow and water (control), or 15% (w/v) ethanol solution, or 15% ethanol solution + 3-HP (15 mg of 3-HP was combined with each 100 ml of ethanol solution) for a period of 5 months. There was also a group of intact 15-month-old mice. Each group consisted of 12 animals. The mean water or ethanol consumption per day was 4-5 ml. Thus one mouse consumed approximately 0.56-0.75 ml/mouse/day of absolute ethanol and/or 0.60-0.75 mg/mouse/day of 3-HP (20-25 mg/kg/day). The mice were tested in shuttle-box two weeks after withdrawal of the experimental diets. The conditioned reflex of active avoidance was developed in mice for 6 days with 40 daily trials for each mouse.

The observation of lipofuscin accumulation in brain of alcohol-treated mice and influence of 3-HP on this age-pigment was performed as described [21].

An experimental model of PS was elicited in rats with injection of MPTP in dose of 10 µg, and MPP⁺ in dose of 5 µg into the *substantia nigra*. Acute and subchronical injection of MPTP (intraperitoneal) were also made. These drugs were synthesized by V.A. Zagorevsky and L.M. Sharkova (Institute of Pharmacology, Academy of Medical Sciences of USSR). Using conventional techniques 1 µl of MPTP or MPP⁺ (or vehicle) were delivered bilaterally from a Hamilton syringe into the *substantia* (AP-4, L-2, H-7,6), according to the stereotaxic atlas of Bures *et al.* [22]. The development of PS was followed by evaluation of motor disorders (oligokinesia, rigidity, tremor). The rigidity and tremor were evaluated by means of a rating scale. Statistical analysis was performed according to Mann-Whitney, Student's t-test.

Results

Experiment I. - Testing of age-dependence factors effect upon retention process revealed a rapid forgetting of CRPA in old (24 months) animals after a one-trial learning procedure. 3-HP administration (40-75 mg/kg daily in drinking water) contributed to memory maintenance expressed in a better conditioned reflex performance in old animals receiving the drug. The retention of CRPA performance remained high in these rats on the 7th day of testing (Fig. 1).

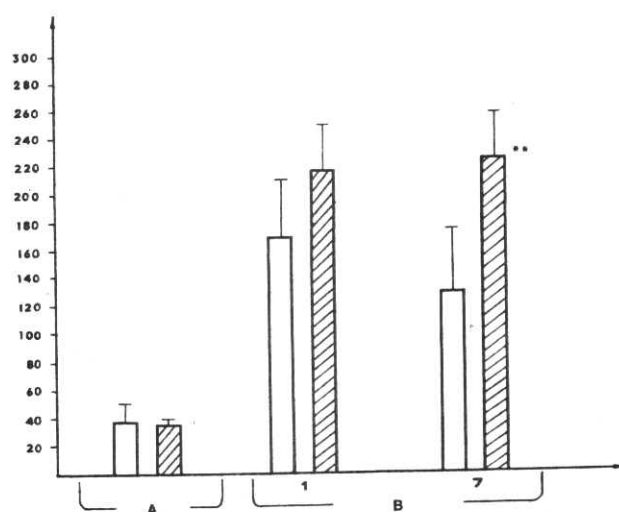


Fig. 1. - The retention test of the conditioned reflex of passive avoidance (CRPA) in old rats.

On the horizontal axis, the days of CRPA learning; on the vertical axis, the time spending in the light compartment of the camera. A: before one-trial learning procedure. B: after learning. Light columns - 24-month-old rats (control), shaded columns - 3-HP-treated 24-month-old rats. ** $p < 0.01$.

Experiment II. - The ability to learn the conditioned reflex of active avoidance (CRAA) in old 24-month animals was assessed using the shuttle-box test. 3-HP was found to increase learning ability in old rats. The treated old rats demonstrated a higher percentage of conditioned reflex responses on the 3th, 4th, 5th and 6th days of learning when compared to the control rats (Fig. 2). This effect was similar to that of nootropics such as piracetam and piritinol upon learning and retention [23, 24].

Experiment III. - In the second series of experiments the model of accelerated aging was employed, using 3-month-old mice exposed to chronic ethanol treatment. Long-term (5 months) consumption of 15% (w/v) ethanol instead of water affected the learning of the CRAA in shuttle-box test. Mice that had consumed ethanol and 3-HP in this test performed better than the "only alcohol"-treated animals (Fig. 3). Chronic alcohol treatment of animals in this model was accompanied by an accelerated deposition of aging pigment (lipofuscin) in ethanol-treated mice brain when compared to that in control animals of the same age and sex. 3-HP was found to prevent lipofuscin accumulation in animals exposed to chronic alcohol administration (Table 1).

Experiment IV. - Extrapyramidal disorders after systemic administration of MPTP was dependent on age of animals, dose of MPTP and duration of the administration. A single intraperitoneal administration of MPTP in doses of 5-10 mg/kg produced slight disorders in some rats. Immediately after administration of MPTP in doses of 30, 40 and 60 mg/kg "Straub tail" phenomenon, tremor and seizures were observed, the latter lasting 50-60 min;

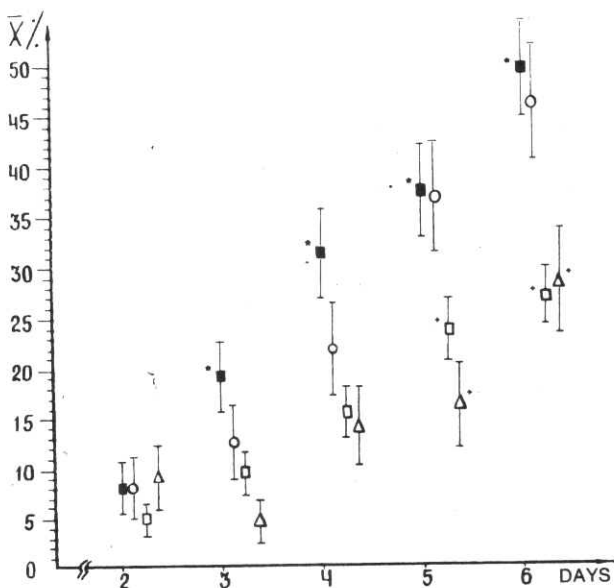


Fig. 3. - Ethanol-induced impairment of learning ability in mice in shuttle-box test.

On the horizontal axis, the days of learning; on the vertical axis, percentage of CRAA. Light circles: untreated 8-month-old mice, light squares - ethanol-treated 8-month-old mice; dark squares ethanol + 3-HP-treated 8-month-old mice; light triangles: untreated 15-month-old mice. *: $p < 0.05$ compared with ethanol-treated mice; +: $p < 0.05$ compared with untreated 8-month-old mice.

extrapyramidal disorders ensued 2-3 h later. After repeated administration the effect of MPTP increased. The effect of systemic administration of MPTP increased also with the age of animals (Table 2).

The stereotaxic administration of MPP^+ (5 μ g) into *substantia nigra* of rats was more effective than MPTP (10 μ g). Rats receiving 5 μ g of MPP^+ intranigally exhibited severe and long-lasting extrapyramidal disturbances. Maximum disturbances were observed 24 h after injection of neurotoxin. During this phase the rats were unable to move and showed both rigidity and tremors (Table 2).

The intranigral administration of MPTP (10 μ g) produced slight disorders, whose peak was observed 4 h after the administration.

Table 1. - Effects of 3-HP on lipofuscin accumulation in mice

Groups of animals	Number of animals	Weight of brain (mg)	Fluorescence /brain
Control (8 months)	13	465.0 \pm 9.8	34.3 \pm 1.5
Control (15 months)	8	462.0 \pm 13.5	121.5* \pm 5.3
Ethanol (8 months)	10	470.8 \pm 8.3	114.8* \pm 7.5
Ethanol + 3-HP (8 months)	11	469.9 \pm 11.1	47.5 \pm 4.5

* $p < 0.001$

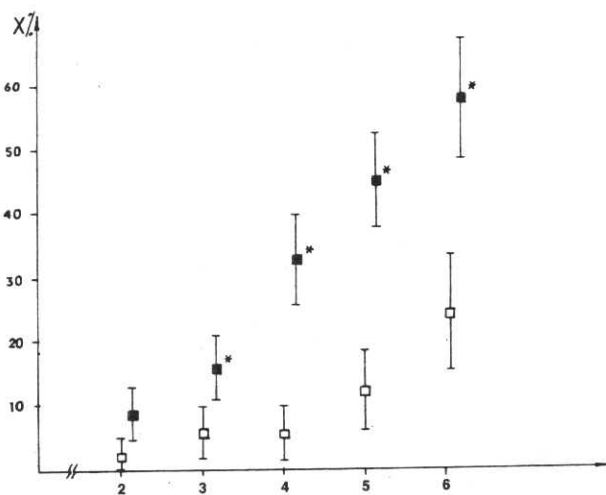


Fig. 2. - The ability to acquisition of the conditioned reflex of active avoidance (CRAA) in old rats.

On the horizontal axis, the days of learning; on the vertical axis, percentage of CRAA. Light squares: control rats (24 months); dark squares: 3-HP-treated rats (24 months). *: $p < 0.01$.

Table 2. - *Effects of MPTP and age of rats, doses and route of administration*

Symptoms of PS	Intranigral administration MPP ⁺ MPTP		Intraperitoneal administration of MPTP						
			Single dose (mg/kg)						Repeated doses (3)
			Age (months)						
	5 µg	10 µg	5	10	30	60	30	15	6
									30 mg/kg
Oligokinesia	+	+	—	+	—	+	+	+	+
Rigidity	+	+	—	—	+	+	+	+	+
Tremor	+	—	—	+	—	+	+	+	+
Exophthalmus	+	—	—	+	+	+	+	+	+
Retropulsion	+	—	—	—	+	+	+	+	+
Salivation	+	—	—	—	+	+	+	+	+
Seizure	+	+	—	+	—	+	+	+	+
Lethality (%)	—	—	—	—	8.4	33.3	—	—	33.3

Discussion

The free radical hypothesis is one of the most accepted theories of aging [3] which proposes that free radicals peroxides damage subcellular enzymes, membranes and nucleic acids. Levels of natural lipid antioxidants do not increase during aging whereas increasing levels of toxic compounds together with relatively decreasing level of regulatory enzymes and natural antioxidants might result in age-related changes.

It can be suggested that lipid peroxidation provoked by free radicals causes the death of nigral dopamine containing neurons in Parkinson's disease [25]. Nigral glutathione and glutathione peroxidase protective mechanisms against the insult become less potent in Parkinson's disease [26]. Besides MPTP and MPP⁺ may kill nigral dopamine containing cells by free-radical-induced lipid peroxidation [27].

These data suggest that basal lipid peroxidation may be increased in Parkinsonian *substantia nigra*. It is possible that nigral dopamine-containing cells in Parkinson's disease undergo enhanced free-radical attack by endogenous neurotoxic species or that normally efficient protective mechanisms fail to prevent lipid peroxidation.

3-HP acts like scavenger of free radical, inhibitor of lipids oxidation of membrane, changes membrane phospholipid composition, fluidity, permeability of membrane and functions of membrane-related enzymes and receptors [4, 5].

In addition, 3-HP was shown to prolong animals life span [1]. Taking into consideration these data it may be suggested that anti-free-radical and membrane modulating properties of 3-HP are responsible for the beneficial effects of these drugs upon age-dependent disturbances of memory and Parkinson's syndrome.

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