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Research report

Long term exposure to norharman exacerbates 6-hydroxydopamine-induced parkinsonism: Possible involvement of L-type Ca^{2+} channels

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ABSTRACT

β-Carbolines (BCs) are considered as endogenous neurotoxins that may contribute to the pathogenesis of Parkinson's disease (PD). However, several lines of evidences show that these compounds have neuroprotective effect. This study was designed to assess effect of long term exposure to norharman, a BC compound which in mammalian brain occurs at high levels in the substantia nigra, on the progress of parkinsonism induced by 6-hydroxydopamine (6-OHDA). Animals were daily treated by norharman at doses 100, 200 and 1000 μg/kg (i.p.) just before to four weeks after the intrastriatal injection of 6-OHDA. Statistical analysis of apomorphine-induced rotation tests demonstrates that treatment by norharman at doses 200 and 1000 μg/kg for four weeks exacerbates significantly behavioral symptoms of the parkinsonism. To explore mechanisms by which norharman affects nigral dopaminergic cells, we studied the role of L-type Ca^{2+} channels. For this purpose, animals were daily treated with either L-type Ca^{2+} channel blocker of nifedipine at doses 2 and 5 mg/kg (i.p.) or nifedipine together with norharman before to four weeks after the 6-OHDA injection. While treatment with nifedipine improved behavioral symptoms of the parkinsonism, treatment with both nifedipine and norharman had no affect on these symptoms. This data indicates that long term exposure to BCs promote nigral dopaminergic cell death possibly through L-type Ca^{2+} channels.

1. Introduction

Norharman is a kind of β-carbolines (BCs) that are heterocyclic indole alkaloids found in grilled meat, alcoholic beverages, brewed coffee, tobacco smoke and in medicinal plants like Peganum harmala [10,11,27,32]. BCs have also been isolated from human body fluids and tissues, e.g. blood platelets, milk, urine, brain, liver, kidney and lens [9]. A wide spectrum of pharmacological actions, including monoamine oxidase inhibition [1], binding to benzodiazepine receptors [2], convulsive or anticonvulsive actions [18], tremorogenesis [19], anxiolytic and behavioral effects [1] antioxidative action [34] and immunomodulatory effects [29] have been attributed to these compounds.

BCs are structurally similar to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and a large body of evidences indicate that N-methylated BCs share several functional and toxic properties with MPP^+, the active metabolite of MPTP [7,21]. They are substrates for the dopamine transporter and inhibit dopamine uptake [31], inhibit mitochondrial respiration at complexes I, II and III [5] and are toxic in various cell culture models [3,6,25,31]. The levels of BCs in plasma and cerebrospinal fluid of patients with Parkinson's disease (PD) have been found are higher than in control groups [15]. Therefore, a possible role of BCs in the pathophysiological processes that initiate PD has been suggested. However, several reports have shown that BCs have effective antioxidant abilities and thus can protect neurons against neurotoxins. BCs inhibit lipid peroxidation of liver microsomes [34], and to attenuate oxidative damage of hyaluronic acid, cartilage collagen and immunoglobulin G [13]. It has been demonstrated that BCs attenuate the cytotoxic effect of glutamate on mouse hippocampal cells [20] and have a protective effect against MPTP-induced neurotoxicity in the mouse [17].

This study was designed to test effect of chronic administration of norharman, a BC compound which in mammalian brain occurs at high levels in the substantia nigra [25], on the progress of parkinsonism induced by 6-hydroxydopamine (6-OHDA). Also, although possible role of BCs in PD is well documented, the mechanisms that by which these compounds affect dopaminergic nigral cells are not clear. Ca^{2+} has been strongly implicated in induction of apoptosis and regulation of the apoptotic signaling pathways [35]. Furthermore, L-type Ca^{2+} channels underlying autonomous activity in dopaminergic neurons [33] and it has been found engagement of L-type Ca^{2+} channels during autonomous pacemaking renders

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nigral dopaminergic neurons susceptible to toxins inhibiting mitochondrial respiratory chains [4]. Therefore, in the second part of this study, we assessed effect of L-type Ca^{2+} channel blocker of nifedipine on the both 6-OHDA induced parkinsonism and effect of norharman on the progress of PD.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Razi Institute, Karaj, Iran), weighing 220–280 g at start of study were housed three–four per cage in a temperature-controlled colony room under light–dark cycle with food and water ad libitum. They were held in their cages 10 days before the experiments. All procedures of this study were according to the guidelines of animal experiments of Research Council at Qazvin University of Medical Sciences.

All animals received 6-OHDA (see below). They were divided in 9 experimental groups: one control group which received just 6-OHDA, two sham-operated groups which received isotonic saline or ethanol as vehicle solution, three NH groups which were treated by norharman at doses of 100, 200 and 1000 μg/kg and two NF groups which were treated by nifedipine at doses of 2 or 5 mg/kg. Vehicle solutions or drug-containing vehicle solutions were intraperitoneally injected daily just before the surgery to fourth week post-surgery.

2.2. Surgical procedures

Unilateral intrastriatal 6-OHDA injection was performed through a 10-ml Hamilton syringe on anesthetized (ketamine 100 mg/kg and xylazine 5 mg/kg, i.p.) rats using stereotaxic apparatus (Stoelting, USA) at the coordinates: L +3 mm, AP +9.2 mm, from the center of the interaural line, and V +6 mm from the surface of skull according to the atlas of Paxinos and Watson [26]. 6-OHDA was dissolved in isotonic NaCl solution containing 0.2 mg/ml of ascorbic acid and was administrated in a single injection of 6 μl (2.5 μg of 6-OHDA in 1 μl) over an 8-min period. At the end of injection, the needle was left in place for an additional 5 min and then withdrawn at a rate of 1 mm/min.

2.3. Behavioral testing

The animals were tested for rotational behavior by apomorphine hydrochloride (0.5 mg/kg, i.p., dissolved in saline) before the surgery and in the second and fourth weeks post-surgery. The rotations were measured according to a method as described previously by Fujita et al. [8]. Briefly, the animals were allowed to habituate for 10 min and then 1 min after the injection of apomorphine, full rotations was counted in a cylindrical container (at a diameter and height of 28 and 38 cm, respectively). Full rotations were counted at 10-min intervals for 1 h in a quiet isolated room. The number of contralateral and ipsilateral rotations (far away and toward the lesioned side, respectively) was counted as positive and negative scores and net number of rotations was defined as the positive scores minus the negative scores.

2.4. Statistical analysis

All data were expressed as mean ± standard error of the mean (S.E.M.). For the apomorphine-induced rotational behavior between the groups, one-way analysis of variance (ANOVA) and Kruskal–Wallis test were used. Within each group and control versus sham groups or drugs-treated versus sham groups, *t*-test, paired *t*-test and Wilcoxon Signed Ranks Test was used. The criterion for statistical significance was $P < 0.05$.

2.5. Drugs

6-OHDA, apomorphine, norharman and nifedipine were purchased from sigma. Ethanol and L(+)-ascorbic acid were purchased from MERCK. 6-OHDA and apomorphine were prepared freshly. Norharman and nifedipine were dissolved in normal saline and ethanol, respectively in concentrations in which volume of intraperitoneal injections was not more than 0.1 ml.

3. Results

Before the surgery, animals showed a few rotations in response to apomorphine administration (Table 1). There was no significant difference in these rotations between all groups. However, most of the rats showed high numbers of apomorphine-induced net contralateral rotations in the second and fourth weeks post-surgery. Only rats responding with >30 net contralateral rotations post-surgery were selected for analysis of the drugs effects. Beside nifedipine groups, in all other groups, number of net contralateral rotations in the fourth week was significantly higher than it in the second week. Because there were no significant differences between control and sham groups, statistical analysis were made between drug treatment groups and sham groups.

Table 1
Net number of presurgery rotations in 60 min period in response to apomorphine administration in all experimental groups. Negative and positive values indicate turns ipsilateral and contralateral to the side of lesion respectively. *n*: number of rats in each group, NH: norharman, NF: nifedipine.

Groups	*n*	Rotations
Control	11	−3.18 ± 0.63
Saline (vehicle)	10	−2.1 ± 0.57
Ethanol (vehicle)	16	1.75 ± 0.83
NH 100	10	−1.3 ± 0.85
NH200	15	−1.53 ± 0.35
NH1000	16	0.0625 ± 0.22
NF2	11	0.545 ± 0.9
NF5	15	−2.2 ± 0.95
Nor + NF	11	0.545 ± 0.275

3.1. Effect of norharman on the apomorphine-induced rotations

Fig. 1 summarizes effect of norharman (NH) at doses of 100, 200 and 1000 μg/kg (i.p.) on the apomorphine-induced rotations in the second and fourth weeks post-surgery. In compare to vehicle group, treatment with norharman at doses of 200 and 1000 increased significantly total net number of contralateral rotations in the fourth week. Number of net contralateral apomorphine-induced rotations increased by 78 and 108% in NH200 and NH1000 groups respectively. Also, number of the rotations in these groups was increased in the second week by 30 and 38% but it was not significant.

3.2. Effect of nifedipine on the apomorphine-induced rotations

Fig. 2 shows effect of nifedipine (NF) at doses of 2 and 5 mg/kg on the apomorphine-induced rotations in the second and fourth weeks post-surgery. In compared to vehicle group, number of net contralateral rotations in the fourth week in both NF2 and NF5 groups was decreased by 50 and 56% respectively, which was statistically significant too. Also and in contrast to other groups, in NF groups net number of contralateral rotations in fourth week was lower than it in the second week.

3.3. Effect of norharman + nifedipine on the apomorphine-induced rotations

There was no significant difference in the apomorphine-induced contralateral rotations in the second and fourth weeks post-surgery between rat group treated with both norharman (1000 μg/kg) and nifedipine (5 mg/kg) and vehicle (ethanol) group (Fig. 3).

4. Discussion

While BCs are considered as endogenous neurotoxins initiating idiopathic PD, there are several evidences indicating BCs can protect neurons against neurotoxicants. This study was designed to assess effect of chronic exposure of BCs on the progress of PD in the 6-OHDA model. Among BCs, we chose norharman because in mammalian brain it occurs at relatively high levels in the substantia nigra [25]. Also, a higher CSF level of active metabolite of norharman has been found in the patients with PD [15]. Our data demonstrate that long-lasting exposure to norharman exacerbates behavioral symptoms of PD. To explore mechanisms mediating norharman effect, we studied the role of L-type Ca^{2+} channels. Chronic treatment with L-type Ca^{2+} channel blocker of nifedipine per se, attenuated behavioral symptoms of PD. However, co-treatment norharman with nifedipine did not have significant effect indicating L-type calcium