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Intracerebral injection of low amounts of norharman induces moderate Parkinsonism-like behavioral symptoms in rat

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ABSTRACT

β-Carbolines (BCs) are considered to be endogenous toxins and have been proposed as possible causative candidates inducing Parkinson's disease (PD). However, there is controversy about the effect and also effective dose of these compounds in the etiology of PD. This study was designed to further examine the effect of norharman (NH), a BC which in mammalian brain occurs at high levels in the substantia nigra, on the development of Parkinsonism-like behaviors in rats. A small amount (4 μl) of NH solution at 2 or 200 ng/ml was unilaterally injected into either striatum or substantia nigra (SN) by stereotaxic surgery. The development of Parkinsonism was assessed by three conventional behavioral tests, compared to the effects of unilateral 6-hydroxydopamine (6-OHDA) — induced lesions in the nigrostriatal pathway. An apomorphine-induced rotational test revealed no Parkinsonism-like behavior in the NH treated groups. However, rats that received the high concentration of NH into their SN showed significant biased swings in the elevated body swing test. In a rotarod test, NH treated groups showed relatively weak motor performance and their learning patterns were close to that of the 6-OHDA treated rats. Considering that the rotational test is only valid in animals with severe Parkinsonism, but time spent on the rotating rod correlates inversely with severity of Parkinsonism, our results indicate that a single exposure to low amounts of NH is effective in producing moderate Parkinsonism-like behavioral symptoms, possibly through a neurotoxic effect of this agent on the SN dopaminergic neurons.

1. Introduction

The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) led to the hypothesis that Parkinson's disease (PD) may be initiated or precipitated by environmental or endogenous toxins through the mechanism(s) similar to that of MPTP in genetically predisposed individuals. Among potential neurotoxicants, β-carbolines (BC) have received the most interest because they occur ubiquitously in our environment and diets, but are also formed endogenously in the human body (Collins and Neafsey, 2002; Herraiz, 2002; Matsubara et al., 2002; Pfau and Skog, 2004; Hamann et al., 2006). BCs structurally resemble MPTP and it has been shown that their N-methylated forms share several functional and toxic properties with MPP^+, the active metabolite of MPTP (Collins and Neafsey, 2002; Matsubara et al., 2002). For example, they induce oxidative stress and inhibit mitochondrial respiration at complexes I, II and III. Several experiments on various cell culture models have confirmed the toxicity of BCs (Collins et al., 1996; Bonnet et al., 2004; Storch et al., 2004; Pavlovic et al., 2006).

A large body of evidence indicates that BCs are involved in the neuropathogenesis of idiopathic PD (reviewed in Collins and Neafsey, 2002; Matsubara et al., 2002). Norharman (NH), a kind of BC which in mammalian brain occurs at high level in the substantia nigra (Pavlovic et al., 2006), could be an underlying factor in the development of PD. NH is methylated to form 2,9-$MeNH^+$ in vitro and in vivo, and a higher cerebrospinal fluid (CSF) level of 2,9-$MeNH^+$ has been found in the CSF of patients with PD (Matsubara et al., 1995). Consistent with this, it has been reported that systemic administration of NH or 2-methylated NH decreases the number of tyrosine hydroxylase positive neurons in the SN and provokes parkinsonian behavior in mice (Matsubara et al., 1998; Ostergren et al., 2006). In fact, an unrecognized parkinsonian model was produced by subchronic intraperitoneal treatment of C57/Bl mice with these compounds (Matsubara et al., 1998).

On the other hand, there is also evidence that BCs may play a neuroprotective role (Tse et al., 1991; Maher and Davis, 1996; Lee et al., 2000; Kim et al., 2001; Splettstoesser et al., 2005). For example, it has been shown that NH inhibits monoamine oxidase (MAO) enzyme (May et al., 1991; Herraiz and Chaparro, 2005) and protects

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dopaminergic neurons against oxidative dopamine metabolism. NH has been isolated from cigarette smoke and has been suggested to be responsible for the lowered activity of MAO enzymes and the observed reduced risk of developing PD in smokers (Rommelspacher et al., 2002; Herraiz and Chaparro, 2005).

N-Methylated β-carbolinium cations are substrates for dopamine transporter (DAT) and therefore DAT-expressing cells are predicted to be more susceptible to N-methylated β-carbolinium cation-mediated toxicity (Storch et al., 2004). Moreover, BCs have a high affinity binding to melanin and long term retention in pigmented tissues confirming their toxic relevance to neuromelanin-containing dopaminergic neurons (Ostergren et al., 2004). Nevertheless, the high selectivity of MPP^+ towards nigrostriatal neurons is neither displayed by 2,9-$Me2NH^+$ nor by other BCs (Matsubara et al., 1998). It has been reported that in rat primary mesencephalic cultures there is virtually no difference in the toxicity towards dopaminergic and GABAergic neurons by 2,9-$Me2NH^+$ (Collins et al., 1996). In these cultures, 2,9-$Me2NH^+$ was moderately selective for dopaminergic neurons only at low concentrations (1 μM), but exerted general neurotoxicity at higher (10 μM) concentrations (Matsubara et al., 1998).

In our lab, several experiments have been conducted to examine the role of NH in PD. Our findings show that long-term exposure to NH exacerbates 6-OHDA-induced Parkinsonism in rat (Haghdoost-Yazdi et al., 2010) indicating that neurotoxicity but not neuroprotectivity is the true effect of NH. We also found that daily treatment of rats with intraperitoneal administration of NH at doses as high as 1 mg/kg cannot produce parkinsonian behavior (Haghdoost-Yazdi et al., 2011). Considering the extensive presence of BCs in our environment, this study was designed to extend our information about the possible Parkinsonism-inducing effect of these compounds. The current paper describes the effect of a single injection of low amounts of NH, this time into the brain itself, on the development of parkinsonian-like behavior in rat.

2. Materials and methods

2.1. Animals and experimental groups

Adult male Wistar rats (Razi Institute, Karaj, Iran), weighing 220–300 g at the beginning of the study, were housed in large cages (38 cm × 59 cm × 20 cm) in a temperature-controlled colony room under light/dark cycle with free access to tap water and standard food. All procedures carried out in this study were in accordance with the guidelines of animal experiments of Research Council at Qazvin University of Medical Sciences.

Rats were divided into nine experimental groups: one untreated group which received neither 6-OHDA nor NH, two control groups that received 6-OHDA (Sigma) into either the striatum (str) or medial forebrain bundle (MFB), SN 2 and SN 200 groups that received NH (Sigma) at concentrations of 2 and 200 ng/ml, respectively, into the substantia nigra (SN), str 2 and str 200 groups that received NH at concentrations of 2 and 200 ng/ml, respectively, into the striatum, and sham SN and sham striatum groups that received the vehicle of NH (ethanol) into either the SN or striatum. The number of animals in each group was eight.

2.2. Experimental design

Except for the untreated group, all animals were subjected to stereotaxic surgery and received either NH, 6-OHDA, or the vehicle. An apomorphine-induced rotational test and an elevated body swing test (EBST) were performed at four time points: before the surgery, and within the second, fourth and eighth weeks post-surgery (Fig. 1). Animals showing net clockwise or counterclockwise rotations less than 10 rotations/h in the presurgery test were selected for experiments. Behavioral tests were performed in several sessions post-surgery because it has been shown that behavioral symptoms of 6-OHDA-induced Parkinsonism change as a function of time (Yuan et al., 2005). Also, it has been reported that repeated assessment is very important for some behavioral tests in rat models with hemiparkinsonism (Borlongan and Sanberg, 1995). A rotarod test was performed in the ninth week post-surgery (Fig. 1).

2.3. Surgical procedures

Rats were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/ kg). Four microliters of NH was unilaterally injected at the rate of 1 μl per min into either the left striatum or left SN according to the atlas of Paxinos and Watson (2007) using stereotaxic apparatus (Stoelting, USA) and through a 10-μl Hamilton syringe. References were bregma in the lateromedial (L) and anteroposterior (AP) axes and the surface of skull for vertical axis. Coordinates for SN were: L +2, AP −5 and V +8.2. For striatum, NH was injected at two sites with coordinates L +2.5, AP +2 , V +7 and L +3.5, AP +0.2, V +6. For the positive control groups, 6-OHDA was dissolved in isotonic NaCl solution containing 0.2 mg/ml of ascorbic acid, and was injected at a single dose of 4 μl (4 μg of 6-OHDA in 1 μl) into the left MFB with coordinates of L +1.2, AP −4.4, V +7.8, or the left striatum with coordinates similar to the site of NH injection. 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.

2.4. Behavioral testing

2.4.1. Elevated body swing test

The elevated body swing test was performed before the apomorphine-induced rotational test according to a slightly modified method as described before (Borlongan et al., 1995). Briefly, the animal was placed in a cylindrical container, and allowed to habituate

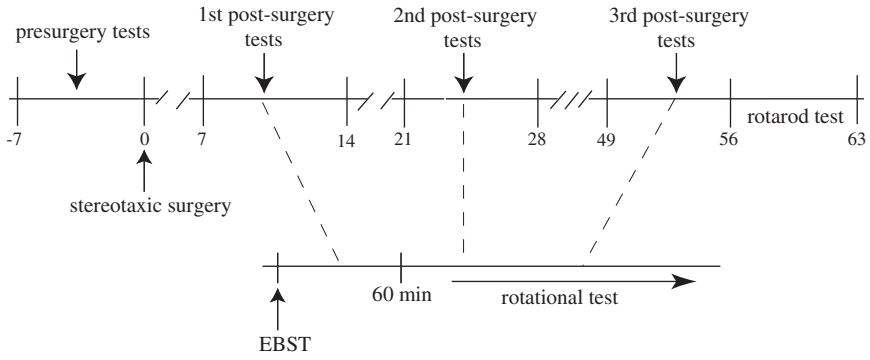

Fig. 1. Time schedule used for animal experiments. Animals were tested by apomorphine-induced rotational test and elevated body swing test (EBST) at four time points: in the week prior to the stereotaxic surgery and in the second, fourth and eighth weeks after that. The rotational tests were performed at least 1 h after termination of the EBST. Performance on the rotating rod was tested in the ninth week post-surgery.