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Age-specific effects of 6-hydroxydopamine lesions of the rat medial prefrontal cortex on stress-induced *c-fos* expression in subcortical areas

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ABSTRACT

As adolescence is a critical period when dopaminergic neuronal maturation peaks, we hypothesized that 6-hydroxydopamine (OHDA) lesions of the medial prefrontal cortex (mPFC) in adolescent rats would have more negative effects than lesions in adult rats. Therefore, we investigated the effects of 6-OHDA lesions of the mPFC in adolescent and adult rats on stress-induced *c-fos* expression in the brain. Adolescent and adult Sprague–Dawley rats, aged 4 and 7 weeks on arrival, respectively, were studied. 6-OHDA (8.0 μg) for the lesion groups and ascorbic acid for the sham groups were injected bilaterally into the mPFC. All animals were pretreated with desipramine 30 min before being anesthetized. The control group did not undergo any surgery-related procedure except the desipramine injection. After recovery for 1 week, the rats were subjected to restraint stress for 1 h. Immediately after the stress, the rats were killed and *c-fos* immunohistochemistry was examined. The *c-fos* expression in the nucleus accumbens core (AcbC), nucleus accumbens shell (AcbSh), CA1, CA3, dentate gyrus (DG), central amygdaloid (Ce), basolateral amygdaloid (BL), and temporal cortex (Tc) was compared. Adolescent rats with 6-OHDA lesions subjected to restraint stress had greater *c-fos* expression in the AcbC, AcbSh, DG, Ce, BL, and Tc, compared to the sham and control groups, whereas these differences were not observed among the adult groups. These results suggest that a hypodopaminergic state in the mPFC of adolescent rats, but not adult rats, is related to increased sensitivity to stress, suggesting that damage to or maldevelopment of dopaminergic neurons during adolescence has an age-specific effect. Further research is warranted to investigate the mechanism of the age-specific effect of 6-OHDA lesions of the mPFC.

1. Introduction

The prefrontal cortex (PFC) plays significant roles in a variety of cognitive functions, including attention, memory, abstract thinking, and action planning. Dopamine (DA) in the PFC is one of the neurotransmitters implicated in maintaining these functions. DA depletion in the medial prefrontal cortex (mPFC) in rats and monkeys caused by 6-hydroxydopamine (OHDA), a selective neurotoxin for DA terminals, has been reported to impair the acquisition of delayed alternation tasks (Bubser and Schmidt, 1990) and spatial delayed response performance (Roberts et al., 1994). DA hypofunction in the PFC is closely associated with the psychopathology of schizophrenia, especially the negative symptoms (Davis et al., 1991). The PFC is also regarded as a crucial area for mediating physiologically adaptive changes promoted by stress. Direct evidence for the involvement of this brain region in stress regulation comes from lesion studies demonstrating that damage to the mPFC results in altered hypothalamic–pituitary–adrenal (HPA) axis responses to stress (Diorio et al., 1993; Sullivan and Gratton, 1999). 6-OHDA lesions of the mPFC in rats have been reported to alter the behavioral response to stress (Carlson et al., 1996).

In adolescent rats, the PFC DA fiber density (Kalsbeek et al., 1988), DA concentration (Leslie et al., 1991) and DA synthesis and turnover (Andersen et al., 1997) peak, in contrast to the adolescent-associated loss of excitatory drive to the cortex (for review, Spear, 2000). These data suggest that a shift occurs in the relative balance between subcortical and cortical DA systems during adolescence toward the greater predominance of cortical DA. The peak development of DA

Abbreviations: AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; BL, basolateral amygdaloid; Ce, central amygdaloid; DA, dopamine; DG, dentate gyrus; HPA, hypothalamic–pituitary–adrenal; i.p., intraperitoneally; mPFC, medial prefrontal cortex; NE, norepinephrine; OHDA, hydroxydopamine; PFC, prefrontal cortex; Tc, temporal cortex.

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neurons during adolescence in rats may be relevant to higher cognitive development during human adolescence, given the implicated role of DA in cognition. Behavioral experiments in laboratory animals have suggested that stress has age-specific effects in adolescents. In terms of acute stress responses, adolescent rats show more stress-induced immobility than do adults during forced swim testing (Walker et al., 1995). Stone and Quartermain (1998) reported that chronic social stress had a greater impact on adolescent than adult mice, suppressing food intake, body weight gain, and time spent on the open arms of a plus maze in adolescents, but not adults. Therefore, we hypothesized that abnormal DA neuronal development or damage during adolescence in rats may have a greater impact on the stress-induced response in adolescents than in adults. DA terminals in the PFC can be destroyed selectively by injecting 6-OHDA, which has been used extensively to explore behavioral or neurochemical responses to DA depletion, especially in relation to the pathogenesis of schizophrenia. However, the 6-OHDA model has been studied mostly in neonates or adults rather than in adolescents.

Therefore, we investigated whether 6-OHDA lesions of the mPFC in adolescent rats have greater effects on stress-induced *c-fos* expression in subcortical areas compared to adults.

2. Methods

2.1. Animals

Adolescent [postnatal day (pnd) 28] and adult (pnd 49) male Sprague–Dawley rats were obtained from Orient Bio (Seoul, Korea). On arrival, the different-aged rats were housed separately, three per cage. The animals were kept under a controlled 12/12-h light-dark cycle (light from 7:00 AM to 7:00 PM) at a room temperature of 21 ± 1 °C and humidity of 55 ± 5%. Food and water were available *ad libitum*. The experimental procedure was approved by the Animal Care and Use Committee of Chonbuk National University Graduate School of Medicine.

2.2. Surgery

Surgery was performed 7 days after the rats arrived. In an effort to spare noradrenergic terminals from 6-OHDA lesions, all animals were pretreated with the norepinephrine reuptake inhibitor desipramine (25 mg/kg, i.p.) 30 min before being anesthetized with a mixture of ketamine (40 mg/kg; Yuhan, Seoul, Korea) and xylazine (2.7 mg/kg; Bayer, Seoul, Korea). The animals were placed in a David Kopf stereotaxic instrument and 1-mm burr holes were made. A silica probe (Polymicro Technologies, Phoenix, AZ, USA) attached to a 30-gauge stainless steel cannula (Small Parts Inc., Miramar, USA) was lowered to the infusion site stereotaxically. Bilateral injections of 6-OHDA hydrobromide (8.0 μg free base/2 μl of vehicle) for the lesion group or vehicle (0.9% saline containing 0.1 mg/ml ascorbic acid) for the sham group were microperfused into the mPFC at a rate of 0.8 μl/min (0.4 μl/injection) for 5 min using a dual-syringe infusion pump (KD Scientific, Holliston, MA, USA). The coordinates of the mPFC were as follows: for adolescent rats, anteroposteriorly (AP) from the bregma +3.0 mm, laterally (L) from the midline +0.7 and +1.5 mm for the left and right, respectively (10° inclination) and ventrally (V) from the skull −3.5 mm; and for adult rats, AP +3.0 mm, L 0.7 and 1.5 mm for the left and right (10° inclination), and V −3.8 mm (Paxinos and Watson, 1998). The silica probe was left in place for 5 min to allow for diffusion. The control group did not undergo any surgery-related procedure except the injection of desipramine.

2.3. Stress procedures

The rats were given 1 week to recover from surgery, during which time all of the animals were handled daily for five days to avoid non-specific Fos immunoreactivity. All three groups (lesion, sham, and control groups) were restrained by placing individual rats in an acrylic restraint tube (Jeungdo, Seoul, Korea) for 1 h. For adolescent rats, the tube was 3.7 cm dorsal/ventral, 5.1 cm wide, and adjustable in length. Adult tubes were 5.2 cm dorsal/ventral, 7.8 cm wide, and adjustable in length. All tubes were cleaned thoroughly with hot water and detergent after each use. The animals were sacrificed immediately after being restrained. Because stressor-induced Fos expression shows diurnal variation in some brain regions, all experimental manipulations were performed between 9:00 AM and 12 noon.

2.4. Monoamine measurement

The animals were decapitated, and the brains were rapidly removed, frozen on dry ice, and stored at −80 °C until analysis. Serial slices approximately 700 μm thick were made in a cold box at −15 °C. Tissue punches from mPFC and Acb were homogenized and centrifuged (18,000 rpm for 15 min at −4 °C), and aliquots of the supernatant were injected into a high-performance liquid chromatography (LC-10AD, Shimadzu, Japan) column with an electrochemical detector (LC-4C, BAS, USA) to determine the concentration of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and norepinephrine (NE). This method has been described in detail elsewhere (Chung et al., 2004; Ichikawa et al., 2005). The tissue pellet protein content was determined using the modified Lowry method.

2.5. Tissue preparation and immunohistochemistry

After perfusion with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer, the brains were removed and postfixed in 4% paraformaldehyde for 4 h and then placed in a 30% sucrose solution overnight at 4 °C.

Frozen coronal sections (40 μm) were cut on a freezing microtome from bregma 4.7 to −10.3 mm. The sections were transferred to 24-well culture plates containing 0.01 M PBS, washed for 30 min, and then incubated with PBS containing 0.5% hydrogen peroxide for 10 min to remove endogenous peroxidase activity. Free-floating sections were placed in 0.01 M PBS buffer containing 2% goat serum, 0.2% Triton X-100, and 0.1% bovine serum albumin (GS-PBST) for 1 h at room temperature. The sections were incubated for 48 h at 4 °C in the primary antibody to *c-fos* (Calbiochem, A brand of EMD Biosciences, Germany), directed against amino acids 4–17 of *c-fos*, diluted in GS-PBST (1:10,000). The sections were washed twice in PBS and incubated for 1 h with a second antibody (biotinylated goat anti-rabbit IgG) and then incubated in an avidin-biotinylated horseradish peroxidase solution prepared from the kit for 1 h at room temperature. The slices were washed twice in ice-cold PBS, and the antibody reaction was developed with 3,3′-diaminobenzidine (0.05%) and 0.003% hydrogen peroxide in 50 mM Tris HCl (pH 7.4). After several rinses in PBS, the sections were mounted on gelatinized slides, dehydrated through an ethanol gradient, cleared in xylene, and coverslipped with Permount® (MERCK, Darmstadt, Germany).

2.6. Histology and data quantification

The location of the lesion site was verified macroscopically during the brain dissection. All the brains examined showed correct positioning of the injection cannula within the mPFC.

The anterior–posterior coordinates of the sections relative to the bregma were +1.60 mm for the nucleus accumbens core (AcbC) and shell (AcbSh), −2.80 mm for the central amygdaloid nucleus (Ce) and basolateral amygdaloid nucleus (BL), −3.30 mm for CA1, CA3, and the dentate gyrus (DG), and −3.80 mm for the temporal cortex (Tc). Images were captured using Nikon ECLIPSE 80i microscope (Nikon, Japan) with a Coolsnap ES camera (Roper Scientific, Inc. Tucson, AZ, USA) at ×100 magnification. Area dimensions were 500 × 500 μm for