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Rapid Communication

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NEUROTOXIC ACTION OF 6-HYDROXYDOPAMINE ON THE NIGROSTRIATAL DOPAMINERGIC PATHWAY IN RATS SENSITIZED WITH D-AMPHETAMINE

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To determine whether behavioral sensitization produced by prolonged D-amphetamine administration affects susceptibility of nigrostriatal dopaminergic neurons to the neurotoxic actions of 6-hydroxydopamine (6-OHDA), rats were treated daily from the 23 rd day after birth for 11 consecutive days with D-amphetamine (1.0 mg/kg s.c.) or saline. On the last day of treatment, one group primed with D-amphetamine and one control group of rats were tested to confirm behavioral sensitization development. The remaining animals were additionally treated on the 34 th day (one day after the last D-amphetamine injection) with 6-OHDA HBr (300 µg in 10 µl i.c.v., salt form, half in each lateral ventricle) or its vehicle. Four weeks later the levels of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-metoxytyramine (3-MT), as well as 5-hydroxytrypatmine (5-HT) and its metabolite 5-hydroxyindoleacteic acid (5-HIAA) were assayed in the striatum, by HPLC/ED. In rats with behavioral sensitization, 6-OHDA reduced endogenous dopamine and its metabolites content to a comparable degree in comparison to controls. This finding indicates that presumed up-regulation of the dopamine transporter in the behaviorially sensitized rats did not increase the neurotoxicity of a high dose of 6-OHDA.

Key words: *dopamine; behavioral sensitization; d-amphetamine; 6-hydroxydopamine (6-OHDA); lesion; rats*

INTRODUCTION

The neurotransmitter dopamine (DA) plays an important role in the modulation of various forms of animal and human behavior such as movement, motivation and reward (1, 2). The central dopaminergic system has been implicated also in the phenomenon known as behavioral sensitization. This term refers to the enhanced behavioral response to indirect-acting dopamine psychostimulant agonists such as amphetamine and cocaine, or the direct dopamine agonists, quinpirole or apomorphine (2-5). Several studies now demonstrate that sensitization of dopamine receptors by direct or indirect-acting dopamine agonists is expressed as increased locomotor activity and augmented stereotyped behaviors (6, 7). In vivo microdialysis studies showed that intermittent injections of dopaminomimetics produced an enduring enhancement of striatal dopamine release following pharmacological stimulation (e.g., amphetamine challenge), and it is considered that this effect may represent one of the major biochemical indices of sensitization (8-10). The precise mechanism of enhanced dopamine release remains unknown. It is postulated to be a consequence of any of the phenomena: subsensitization of the ventral tegmental area (VTA) presynaptic dopamine D_2 autoreceptors and subsequently increased tyrosine hydroxylase activity, increased sensitivity of dopamine D_1 VTA presynaptic receptors, up-regulation of the dopamine transporter, or supersensitization of postsynaptic dopamine D_2 receptors (4, 5, 11). Such neuroadaptation of the dopaminergic system to subsequent amphetamine treatment may change the neuronal susceptibility to various kinds of neurotoxins, which have deleterious biological consequences (12-14).

6-hydroxydopamine (OHDA) has long been a useful pharmacological tools for producing toxicity in monoaminergic neurons (15). By varing the regimen of 6-OHDA administration, including dosage and site of administration, the relative degree of destruction of dopamine innervation is practicable. Lesions with 6 µg of 6-OHDA into the median forebrain bundle of adult rats produced strong dopamine depletion (78%) while lesions with 2 µg of 6-OHDA produced only partial dopamine depletion (46%) (16). Previously we showed that if 6-OHDA were administered intracerebroventriculary (i.c.v.) to rats up to 10 days after birth, striatal dopamine content could be reduced by as much as 99% (17), but if 6-OHDA were administered i.c.v. to rats at 35 and again at 42 days of their life, dopamine content was reduced by only 37% (18). Several reports demonstrate that there are many other factors such as temperature or hormonal balance that can influence the destructive action of 6-OHDA on dopamine containing neurons (14, 19, 20). Taking these observations into account, and on the basis of the reputed association of behavioral sensitization with up-regulation of the dopamine transporter, we evaluated the susceptibility of the nigrostriatal dopaminergic system to 6-OHDA in animals behavioral sensitized with priming doses (i.e., repeated daily doses) of D-amphetamine.

MATERIALS AND METHODS

Animals

Male Wistar rats were obtained from University Animals Department (Katowice, Poland) and were housed in a well-ventilated room, at 22 ± 2°C under a 12h light:12h dark cycle (lights on 7:00 a.m. to 7:00 p.m.), and with free access to food and water. Rats were weaned at 21days. All procedures, reviewed and approved by the Institutional Animal Care Committee, are in accord with principles and guideline described in the NIH booklet *Care and Use of Laboratory Animals.*

Treatments

All rats were treated with either saline vehicle (1.0 ml/kg, i.p.) or D-amphetamine (1.0 mg/kg, i.p.) (Sigma Chemical Co., St Louis, MO, USA) for 11 consecutive days, from the 23rd to 32nd day after birth. Again, on the 33rd day, a single dose of vehicle or D-amphetamine was administered, to observe acute locomotor effects (see below). On the 34th day rats were anesthetized with ketamine (60 mg/kg, i.m.) (Bristol Labs, Syracuse, NY, USA) and treated with 6-OHDA HBr (300 µg per 10 µl i.c.v., salt form, half in each lateral ventricle) (Sigma) or with the vehicle saline (0.85%) - ascorbic acid (0.1%). Desipramine HCl (20 mg/kg, i.p., base; 1 h) (Sigma) and pargyline-HCl (50 mg/kg, i.p., salt form; 0.5 h) (Sigma) were administered prior to 6-OHDA.

Behavioral observation

On the 33rd day after birth, rats were placed singly in plastic cages (48x26x18 cm) with wood chip bedding, in a quiet and well-lighted room. After 30 min acclimation rats were treated with D-amphetamine (0.5 mg/kg, i.p.) and were individually observed for locomotor activity (walking, running, grooming, sniffing, eating and digging), starting 10 min later, for 10 min periods, every 10 min until 80 min (rats were observed for 40 min in total).

Biochemical assay

Four weeks after 6-OHDA treatment, rats were decapitated and each brain was immediately excised. The striatum was separated and placed on dry ice, weighed and stored at -70°C, pending assay. The samples were homogenized in ice-cold trichloracetic acid 0.1M, containing 0.05 mM of ascorbic acid for about 15 - 20 seconds. After centrifugation (5,000xg for five min), the supernatants were filtred through 0.2µm cellulose membranes (Titan MSF Microspin Filters, scientific resources Inc., Eatontown GB) and supernatants injected onto the HPLC/ED column. Levels of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT); as well as 5-hydroxytrypatmine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were assayed by HPLC/ED (21, 22).

The composition of the mobile phase was: 75mM mM NaH_2PO_4 x $2H_2O$ (Avocado, Research Chemicals Ltd), 1.7 mM 1-octansulphonic acid (Avocado, Research Chemicals Ltd), 5 µM EDTA (Avocado, Research Chemicals Ltd), 100 µl triethylamine (Sigma), 9.5% acetonitril (Lab-Scan), pH 3 adjusted with phosphoric acid (Fluka). The flow rate was 0.7 ml/min, temperature assay 22°C and the oxidize potential was +700 mV, 10nA/V sensitivity. Peaks were automatically integrated by universal chromatographic interface UCI-100 (Dionex, Geramny).

The used instrumentation included: an electrochemical detector Gilson (France) model 141 with flow cell, piston pump Gilson (France) model 302 with head 5SC, manometric module Gilson (France) model 802, thermostat for STH 595 column (Dionex, Geramny), precolumn Hypersil BDS

C18, 10x4 mm, 3µm (ThermoQuest GB) and chromatographic column Hypersil BDS C18, 250x4.6 mm, 3µm (ThermoQuest GB).

Data Analysis

Group differences in locomotor activity were analyzed by student's *t*-test. Group differences in monoamines and metabolites were assessed by an analysis of variance (ANOVA) and the post-ANOVA test of Newman-Keuls. A *P value <0.05 was taken as the level of significant difference.*

RESULTS

D-Amphetamine-induced Behavioral Effects in Primed Rats

In rats treated daily with a 1.0 mg/kg (i.p.) dose of D-amphetamine from the 23rd to the 33rd day after birth (i.e., primed rats), there was greater D-amphetamine-induced locomotor activity on the 34th day after birth at all observation times (10, 30, 50, 70 min) vs. D-amphetamine effects in non-primed rats. Differences between the groups ranged from as much as ~45% greater locomotor time at 10 min to ~10% greater locomotor time at 70 min in the primed rats (*Fig. 1*). These findings demonstrate behavioral sensitization in the primed rats.

6-OHDA Effects on Striatal DA and Metabolites in Primed and Non-primed Rats

As shown in *Fig. 2*, a bilateral i.c.v. injection of 6-OHDA HBr (300 µg; desipramine pretreatment) at 34 days after birth reduced striatal DA content, 4

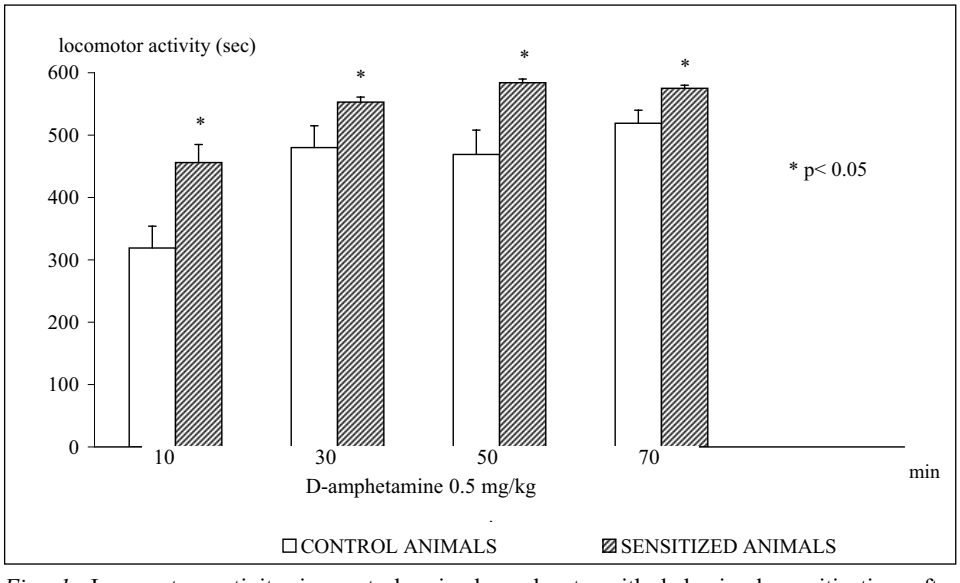

Fig. 1. Locomotor activity in control animals and rats with behavioral sensitization after D-amphetamine challenge 0.5 mg/kg i.p. (n=8)

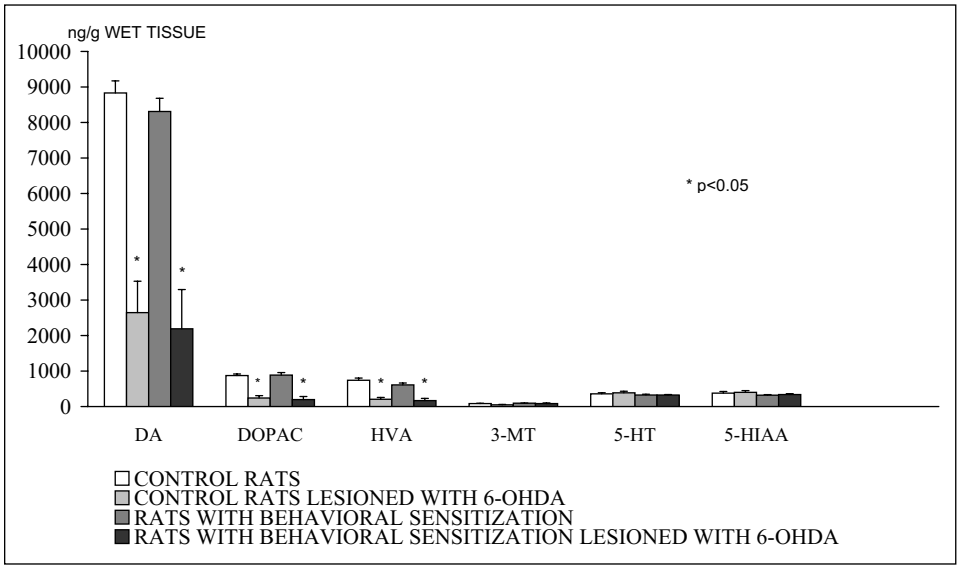

Fig. 2. Effect of 6-OHDA on concentration of DA, DOPAC, HVA, 3-MT, 5-HT and 5-HIAA in striatum of control animals and rats with behavioral sensitization (n=7)

weeks later, by 70-75% in non-primed and in D-amphetamine-primed rats. Near-identical percentage changes in striatal DOPAC and HVA were produced in both groups of rats by 6-OHDA, while 3-MT, 5-HT and 5-HIAA levels remained unaltered by the 6-OHDA treatment. These findings indicate that 6-OHDA did not produce greater destruction in the nigrostriatal terminal region (striatum) in primed vs. non-primed rats.

DISCUSSION

Amphetamine releases dopamine from non-vesicular stores by binding to monoamine transporters as a false substrate, thereby promoting reverse transport of cytosolic dopamine and increasing its extracellular level which in turn produces increased locomotor activity in laboratory animals (23, 24). Several studies demonstrated that repeated treatment of psychostimulants leads to the development of behavioral sensitization, an augmented behavioral response to drug re-administration (4, 10). Findings in the present study, in which we observed enhanced D-amphetamine-induced locomotor activity in rats primed with daily injections of D-amphetamine from the 23rd to the 33rd day after birth, are in accord with prior studies (*Fig. 1*).

Although the precise biochemical mechanisms of behavioral sensitization are not well established, behavioral sensitization is postulated to be a consequence of

changes in reactivity of dopamine receptors as well as up-regulation of the dopamine transporter. For example, Shilling et al. (25) showed that repeated amphetamine treatments resulted in a sensitization response profile to amphetamine challenge; and this was accompanied by up-regulation of dopamine transporter mRNA in both the VTA and substantia nigra. Also, Itzhak and Martin (6) established that cocaine- and ethanol-induced behavioral sensitization is similarly associated with upregulation of striatal dopamine transporters. And Mead et al. (26), assessing the locomotor-stimulant effects of cocaine in dopamine transporter knockout mice, reported that the dopamine transporter is necessary for both the acute locomotor response to cocaine and the subsequent development of sensitization. In further support, Brown et al. (27) reported that a single administration of cocaine and quinpirole but not methamphetamine, affected vesicular monoamine transporter II (VMAT II) which in turn increased intraneuronal vesicular [^{3}H]dopamine uptake. Therefore, on the presumption that the dopamine transporter is key to behavioral sensitization, we expected that up-regulation of the dopamine transporter might increase the susceptibility of dopamine neurons to neurotoxic actions of 6-OHDA. We know that 6-OHDA is accumulated in dopamine nerves via the dopamine transporter and that drugs which block the norepinephrine- or dopamine-transporter prevent 6-OHDA neurotoxicity. Also, 6-OHDA enters vesicles in dopamine nerves via the VMAT II transporter, and can thereby displace dopamine and act as a false neurotransmitter.

Murray et al. (20) demonstrated a sexually dimorphic (male-dominant), dose-dependent susceptibility in rats to 6-OHDA. Following gonadectomy, dopamine depletion resulting from a submaximal dose of 6-OHDA (1 μg) was reduced in male rats, whereas in females, ovariectomy enhanced dopamine depletion. These findings strongly suggest that there are sex differences in the mechanisms whereby nigrostriatal dopaminergic neurones respond to injury. These data are in agreement with other reports e.g., Datla et al. (12), suggesting that estrogen treatment protects the nigrostriatal dopaminergic system to neurotoxic action of 6-OHDA. The most likely explanation of these findings seems to be changes in dopamine transporter functioning, whose density depends on estrogen levels. Also, Van Kampen et al. (14), demonstrated that the dopamine transporter plays a critical role in determining destructive susceptibility to both 6-OHDA and MPP^+ (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium ion).

In rats in which the phosphorothioate antisense oligonucleotide to the dopamine transporter was infused for 7 days into the left pars compacta of the substantia nigra, targeting the mRNA for the dopamine transporter, there was a reduction in [^{3}H]WIN 35-428 binding in left striatum accompanied by a reduction in levodopa- and amphetamine-induced contralateral rotations. In addition, when rats were given unilateral pretreatment with the antisense for the dopamine transporter, bilateral infusion of MPP^+ or 6-OHDA resulted in asymmetrical striatal (3)H-WIN 35-428 binding and dopamine content as well as significant

apomorphine-induced ipsilateral rotations, suggesting neuroprotection of nigrostriatal neurons on the antisense-treated side. Many other factors can change susceptibility of dopamine neurons to the neurotoxic action of 6-OHDA. Transcription factor (Nurr1) that is highly expressed in midbrain dopamine neurons (28), or hypothermia associated with pentobarbital anesthesia, protect dopaminergic neurons from the toxic effects of 6-OHDA in rats (19).

In the present study, as indicated by neurochemical analysis of the striatum, 6-OHDA produced a similar degree of reduction in endogenous dopamine in primed and non-primed rats (68% and 63%, respectively). It is believed that the present study is the first to demonstrate that behavioral sensitization does not change susceptibility of the nigrostriatal system to neurotoxic action of a high dose of 6-OHDA. These results fail to confirm that the dopamine transporter has a critical role in determining susceptibility of dopaminergic nerves to a high dose of 6-OHDA.

However, it must be recognized that there are at least two types of sensitization with distinct neuronal mechanisms. For example, during long-term withdrawal vs. short-term withdrawal from substance abuse (i.e., amphetamine, cocaine), there is accentuated behavioral sensitization (5). In our study we examined rats with short-term sensitization. Further studies are needed to test susceptibility of dopamine-containing neurons to 6-OHDA in rats with long-term sensitization, particularly because molecular changes in the dopamine system are different for each type of sensitization. Finally, it must be considered that the high dose of 6-OHDA used in the present study may have overwhelmed the capacity of dopamine transports to ‘regulate’ uptake (29), and that a low dose of 6-OHDA may produce different results.

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