Behavioural Brain Research 282 (2015) 125–132

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Alterations in brain neurotrophic and glial factors following early age chronic methylphenidate and cocaine administration

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HIGHLIGHTS

- Cocaine administration was associated with increased locomotor activity.
- There are age-dependent alterations in GLT1 and GFAP mRNA expression levels.
- GDNF mRNA levels decrease with age progression.
- GDNF mRNA levels increase after 21 withdrawal days from cocaine and MPH treatments.
- Cocaine or MPH treatments and age affect prefrontal BDNF protein expression.

ARTICLE INFO

Article history:
Received 20 October 2014
Received in revised form
30 December 2014
Accepted 31 December 2014
Available online 8 January 2015

Keywords:
Attention deficit hyperactivity disorder (ADHD)
Cocaine
Methylphenidate (MPH)
Brain derived neurotrophic factor (BDNF)
Glial-cell derived neurotrophic factor (GDNF)

ABSTRACT

Attention deficit hyperactivity disorder (ADHD) overdiagnosis and a pharmacological attempt to increase cognitive performance, are the major causes for the frequent (ab)use of psychostimulants in non-ADHD individuals. Methylphenidate is a non-addictive psychostimulant, although its mode of action resembles that of cocaine, a well-known addictive and abused drug. Neuronal- and glial-derived growth factors play a major role in the development, maintenance and survival of neurons in the central nervous system. We hypothesized that methylphenidate and cocaine treatment affect the expression of such growth factors. Beginning on postnatal day (PND) 14, male Sprague Dawley rats were treated chronically with either cocaine or methylphenidate. The rats were examined behaviorally and biochemically at several time points (PND 35, 56, 70 and 90). On PND 56, rats treated with cocaine or methylphenidate from PND 14 through PND 35 exhibited increased hippocampal glial-cell derived neurotrophic factor (GDNF) mRNA levels, after 21 withdrawal days, compared to the saline-treated rats. We found a significant association between cocaine and methylphenidate treatments and age progression in the prefrontal protein expression of brain derived neurotrophic factor (BDNF). Neither treatments affected the behavioral parameters, although acute cocaine administration was associated with increased locomotor activity. It is possible that the increased hippocampal GDNF mRNA levels, may be relevant to the reduced rate of drug seeking behavior in ADHD adolescence that were maintained from childhood on methylphenidate. BDNF protein level increase with age, as well as following stimulant treatments at early age may be relevant to the neurobiology and pharmacotherapy of ADHD.

1. Introduction

A major concern arising from overdiagnosis of attention deficit hyperactivity disorder (ADHD) [1–4] is the frequent use of psychostimulants in subjects who are not properly diagnosed. Another major problem is the psychostimulants usage as neuroenhancers in non-ADHD patients. As stated by Normann and Berger [5], neuroenhancement is a pharmacological attempt to increase cognitive performance in healthy humans. Methylphenidate, a common treatment for ADHD, is extensively misused, especially by students [6,7].

Psychostimulants are controlled substances, aimed to enhance monoaminergic transmission in the brain. Amphetamine, cocaine and methylphenidate are examples of psychostimulants, who

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http://dx.doi.org/10.1016/j.bbr.2014.12.058
0166-4328/

differ in their mode of action [8–11]. Psychostimulants modulate the monoamine transporters function, thereby increase the monoaminergic signaling, in one of two mechanisms; inhibition of monoamine reuptake, like cocaine, methylphenidate and amphetamine, or substance-type releasers, as amphetamine [12,13]. Methylphenidate is a non-addictive psychostimulant although it is important to mention that its mode of action resembles that of cocaine, a well-known addictive and abused drug. Both methylphenidate and cocaine inhibit the reuptake of dopamine, resulting in an increase in dopamine levels at the synaptic cleft [14]. Dopamine transporter is a known molecular target which mediates the abuse-related effects of cocaine. The ability of cocaine to inhibit dopamine reuptake correlates with its potency to maintain drug self-administration [13].

Neurotrophic factors play a major role in the development, maintenance and survival of neurons in the central nervous system [15,16]. Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family; it is secreted as pro-BDNF and cleaved into its mature form. Its action is mediated through the tyrosine kinase receptor B (TrkB), which leads to a signaling cascade of the ERK pathway. Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor β (TGF-β) superfamily. GDNF is a secreted protein, who acts through the receptor tyrosine kinase, which also leads to the stimulation of ERK pathway [17,18].

In this study we investigated the involvement of neurotrophic factors (BDNF and GDNF), as well as glial parameters, in neuroplasticity following chronic cocaine or methylphenidate treatment in young rats. We hypothesized that chronic exposure of young rats to dopamine stimulation induced by either methylphenidate or cocaine will be associated with increased locomotor activity (as assessed by the open field test) and improvement in spatial working memory (as assessed by the Y maze test), parallel to elevation in the expression of brain BDNF and GDNF.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (SD strain) at the age of 10 days (30 g) with nursing mothers were purchased from Harlan laboratories (Jerusalem, Israel). The rats were housed 10 per cage, with a nursing female, at 22 ± 2 °C and a 12 light:12 dark hours cycle (lights at 05:00 h). At postnatal day (PND) 21 the rats were separated from their nursing mothers and housed four per cage, with unlimited access to commercial pellet food and tap water. All animal procedures were approved by the Animal Care Committees of Tel-Aviv University (approval numbers: M-11-012 and M-13-012).

2.2. Materials

Cocaine hydrochloride was purchased from Sigma–Aldrich (St. Louis, MO, USA). Methylphenidate hydrochloride (Ritalin®) was synthesized by Novartis Pharma AG (Basel, Switzerland). TRIzol was purchashed from Invitrogen, Life Technologies (Carlsbad, CA, USA). Chloroform, iso-propanol, sodium metavanadate, NP-40, glycerol, triton X-100, PMSF, aprotinin and leupeptin were of highest purity available from Sigma–Aldrich (St. Louis, MO, USA). Ultra-pure water (DNAse and RNAse free water) was obtained from Biological Industries (Beit-Haemek, Israel). High capacity cDNA RT kit, fast SYBR green master mix, TaqMan gene expression assays (Assay ID: GDNF: Rn00569510_m1, GAPDH: Rn01775763_g1) and TaqMan gene expression master mix were purchased from Applied Biosystems – Life Technologies (Foster city, CA, USA). Pierce BCA protein kit was purchased from Thermo scientific (Rockford, IL, USA). BDNF and GDNF ELISA kits and Substrate reagent pack (stabilized hydrogen peroxidase and stabilized tetramethylbenzidine) were purchased from R&D systems (Minneapolis, MN, USA). All other chemicals were of highest purity obtainable through regular commercial sources.

2.3. Cocaine and methylphenidate treatment protocols

Beginning on PND 14, three groups of rats ($N = 32$/group) were administered saline, cocaine (15 mg/Kg) or methylphenidate (3 mg/Kg) intraperitoneally for 21 days; drug administration was discontinued at PND 35. The doses of methylphenidate and cocaine were determined according to our previous reports on these agents [19,20]. Eight rats from each treatment group assembled an age group ($N = 24$/age group) and were examined once (at the corresponding age) during the study. There were separate saline control group for each cocaine and methylphenidate conditions. The rats in the first group, at PND 35, were examined behaviorally (open field and Y maze) 20 min after the drugs administration. 24 h later they were sacrificed (decapitation by guillotine) and their brains were dissected on ice. All other animals were sacrificed at three different time points (PND 56, 70 and 90; 24/age group), after behavioral examination.

2.4. Open field

This test was chosen since it is sensitive to changes in locomotor activity. The test was conducted as described previously by Hall [21]. A rat was placed in a 100 cm × 100 cm rectangular black-colored apparatus divided into 20 cm^2, in the middle of one wall near a black-colored box known as home. PND 35 rats were examined 20 min following administration. After 5 min observation and video recording the animal was removed, and the apparatus was cleaned using ethanol and allowed to dry in order to eliminate any odor cues before the recording of the next animal. Activity, zone crossing events, track length and velocity were analyzed using Bio-observe computer software.

2.5. Y maze

This test was chosen since it is sensitive to alteration in spatial working memory. The Y shaped maze was made of three identical black colored arms, each arm of 50 cm long, 20 cm wide and 30 cm height with a 120° angle separating the arms. The test was conducted as described previously by Dellu et al. [22]. The test consisted of two phases separated by a 2 min interval. In the first phase, one arm of the maze was blocked. The rat was placed facing the back wall of one arm, and had 5 min to explore the two unblocked arms. In the second phase the rats had free excess to all arms for another 2 min. The apparatus was cleaned using ethanol and allowed to dry in order to eliminate any odor cues before the recording of each new examined rat. Scoring was calculated as entrance of the rat into each of the arms. Discrimination ratio (relative exploration time spent at the novel arm out of total time spent in the maze) was used to compare between the groups.

2.6. RNA extraction

In order to yield RNA from a brain sample we used the TRIzol reagent, a monophasic solution of phenol and guanidine isothiocyanate, which was first introduced by Chomczynski and Sacchi [23,24]. Following homogenization of the tissue in TRIzol reagent, chloroform was added, and a 15 min 12,000 × *g* centrifugation separated the homogenate into 3 layers. The upper aqueous layer contained RNA. Precipitation of the RNA was made using isopropanol followed by 75% ethanol wash of the RNA pellet formed