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Perspective

Sex-dependent changes in brain CB1R expression and functionality and immune CB2R expression as a consequence of maternal deprivation and adolescent cocaine exposure☆

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ABSTRACT

Early life stress has been associated with several psychiatric disorders, including drug addiction. Actually, maternal deprivation (MD) alters the endocannabinoid system, which participates in motivation and reward for drugs, including cocaine. At youth, the rate of cocaine abuse is alarmingly increasing. Herein, we have investigated the consequences of MD and/or adolescent cocaine exposure in brain CB1Rs and CB2Rs in immune tissues. Control and maternally deprived (24 h on postnatal day, pnd, 9) male and female *Wistar* rats were administered cocaine (8 mg/kg/day) or saline during adolescence (pnd 28–42). At adulthood, [^{3}H]-CP-55,940 autoradiographic binding was employed for the analysis of CB1R density and CP-55,940-stimulated [^{35}S]-GTPgammaS binding for CB1R functionality; CB2R expression was analyzed by Western blotting. Sex differences in CB1R expression and functionality were found, and MD induced important and enduring sex-dependent changes. In addition, the plastic changes induced by adolescent cocaine administration in brain CB1Rs were differentially influenced by early life events. MD increased spleen CB2R expression while adolescent cocaine administration attenuated this effect; cocaine exposure also diminished CB2R expression in bone marrow. Present findings provide evidence for changes in brain CB1R expression and functionality and immune CB2R expression as a consequence of early life stress and adolescent cocaine exposure, and indicate functional interactions between both treatments, which in many regions differ between males and females.

1. Introduction

Early life stress has been extensively associated with adverse physical and emotional outcomes that occur later in life. Increasing literature provides evidence for a link between the exposure to early life stressors and the appearance of clinical symptoms of psychiatric disorders [1,2], including drug addiction [3], and maladaptive immune responses [4]. Similarly, in rodent models, detrimental behavioral, neuroendocrine and immunological sequels have been described as long-lasting consequences of early-life stress. Actually, early maternal deprivation (MD) in rats, a single 24 h episode of mother-litter separation on early postnatal days, has been reported to provoke a deregulation of stress reactivity and immune function together with behavioral alterations that resemble depressive- and psychotic-like symptoms [5–8]. In addition, stress, and also MD, has been demonstrated to significantly affect behavioral responses to different drugs of abuse, including cocaine [9,10]. Cocaine is a potent stimulant drug with highly addictive properties [11,12] that also provokes important immunological alterations among abusers [13,14]. At present, cocaine abuse is particularly high among young adults, whose rate of cocaine consumption was 1.5% in 2010 [15]. Despite the increasing rate of cocaine consumption in the juvenile population, animal studies have scarcely investigated the long-term consequences of cocaine administration during adolescence.

The endocannabinoid (eCB) system is a retrograde lipid signaling system which mainly acts through the activation of G-protein-coupled cannabinoid receptors, with different pharmacological properties and distribution patterns, i.e., cannabinoid

☆ Perspective articles contain the personal views of the authors who, as experts, reflect on the direction of future research in their field.

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receptor type 1, CB1R and type 2, CB2R. CB1R is predominant within the central nervous system, with an abundant presence in brain regions involved in emotional processing, motivation, motor activation and cognitive function [16]. In contrast, CB2R has been classically located in peripheral immune tissues mediating immunosuppression [17]. Exposure to early life stress influence eCB system development. In particular, MD significantly increased the content of 2-arachidonyl glycerol (2-AG), one of the main endogenous ligands [18], possibly by altering the expression of the enzymes responsible for its metabolism [19]. Similarly, the eCB system seems to underlie some of the neurobehavioral effects of cocaine [20]. Genetic and pharmacological blockade of CB1Rs decreased animals' motivation for cocaine self-administration [21,22], CB1Rs appear to mediate cocaine-induced locomotor sensitization [23], as well as cocaine relapse [24,25]. Moreover, chronic cocaine exposure during adulthood induced a decrease in CB1R mRNA levels in certain brain regions, i.e., ventromedial hypothalamic nucleus and cerebral cortex [26] as well as a modest reduction of 2-AG content within the limbic forebrain [27]. Great interest and concern on the investigation of the long-lasting affection of the eCB system due to early life stress and adolescent cocaine exposure has arisen. Therefore, we investigated the long-term effects of early MD in combination with adolescent cocaine exposure on brain CB1R expression and functionality as well as CB2R expression in immune tissues. Since remarkable sex differences have been described regarding the consequences of early life stress [28,29] and also in relation to cocaine consumption patterns [15] and effects [30], male and female animals were considered in the present study.

2. Materials and methods

Experiments were approved by the local Animal Ethics Committee, and were designated and performed in compliance with the Royal Decree 1201/2005, October 21, 2005 (BOE n° 252) about protection of experimental animals, and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.1. Animals

Wistar albino rats of both sexes were used. Subjects were the offspring of rats purchased from Harlan Interfauna Ibérica S.A. (Barcelona, Spain) mated (one male × two females) approximately 2 weeks after their arrival. All animals were maintained at constant conditions of temperature (22 ± 2 °C) and humidity (50 ± 2%) in a reverse 12 h dark/light cycle (lights on at 08.00 p.m.), with free access to food (A04/A03; Safe, Augy, France) and water. On the day of birth (pnd 0), litters were culled and sex balanced to eight pups per dam (four males and four females). At weaning (pnd 22), animals were housed in groups of four siblings of the same sex per cage.

2.2. Maternal deprivation

Maternal deprivation (MD) was performed as previously described [31]. In brief, dams from the MD group were removed from their home cages at pnd 9 (09.00 a.m.) leaving rat pups by their own for 24 h. On pnd 10 (09.00 a.m.), dams were placed back in their corresponding home-cage. Dams from the control group were briefly removed from their home-cages on pnd 9 and 10 to mimic the manipulation submitted to the experimental group.

2.3. Pharmacological treatment

Cocaine hydrochloride (Alcaliber, Madrid, Spain) was dissolved in saline (0.9%) at a final concentration of 4 mg/ml. Given the critical period selected for drug administration (adolescence), and the schedule of drug administration (15 consecutive days), a moderate dose of cocaine, previously reported in literature [32], was selected. In brief, rats received daily intraperitoneal injections of cocaine (Coc, 8 mg/kg), or saline (Sal), at a volume of 2 ml/kg, during adolescence (from pnd 28 to 42).

2.4. Experimental design

Rats were assigned to eight experimental groups (8–16 animals per group) according to (i) sex (males or females), (ii) neonatal manipulation (control or MD) and (iii) adolescent cocaine administration (Sal or Coc). Animals' body weight was registered at both pnd 9 and 10 to control for maternal deprivation effects. Thereafter, body weight was registered daily from pnd 27 to 42 to investigate cocaine effects during treatment; and from pnd 50 to 60 to study cocaine withdrawal effects on body weight gain. Body weight before pharmacological treatment (drug-free state, on pnd 27) was used as the reference value to calculate the percentage of body weight gain for each animal. Once finished the experimental procedures – except for body weight control – animals were left undisturbed and sacrificed by decapitation on pnd 85. Brains were rapidly removed, as well as immune tissues (spleen, thymus and bone marrow), samples were flash frozen in dry ice and stored at −80 °C.

2.5. Autoradiographic-binding studies

Brain coronal sections (20 μm thick) were cut on a cryostat and thaw-mounted on gelatin-coated slides. Sections were then stored at −80 °C until they were processed for autoradiographic-binding studies.

2.5.1. [^{3}H]CP-55,940 receptor autoradiographic-binding

Slides were brought to room temperature, then incubated for 2.5 h at 37 °C with 10 nM [^{3}H]CP-55,940 (Perkin Eilmer Life Sciences, Milan, Italy) in binding buffer (50 mM Tris–HCl, pH 7.4, 5% BSA). Adjacent cerebral sections were incubated in parallel with 10 μM CP-55,940 to assess non-specific binding. Sections were washed for 1 h at 4 °C in 50 mM Tris–HCl, pH 7.4, 1% BSA buffer and again for 3 h in the same conditions. They were then dipped in 50 mM Tris–HCl buffer (pH 7.4, 5 min) to remove excess BSA, dipped 5 min in distilled water and dried under a cool air stream. Autoradiograms were generated by exposing the dried sections for 14 days to Kodak Biomax MR films (Perkin Elmer Life Sciences, Milan, Italy).

2.5.2. CP-55,940-stimulated [^{35}S]GTPγS binding in autoradiography

Slides were incubated in assay buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, 10 mU/adenosine deaminase, 0.1% BSA, pH 7.4) at 25 °C for 10 min and then in 3 mM GDP in assay buffer at 25 °C for 15 min. They were then transferred to assay buffer containing 3 mM GDP and 0.04 nM [^{35}S]GTPgammaS with (stimulated) or without (basal) 5 μM CP-55,940 and incubated at 25 °C for 2 h. Slides were rinsed twice in 50 mM cold Tris–HCl buffer (pH 7.4) and once in deionised water, dried, and exposed to Kodak Biomax MR films (Perkin Elmer Life Sciences, Milan, Italy) for 48 h.

2.5.3. Image analysis

Autoradiographic film intensity was assessed by measuring gray levels with an image analysis system consisting of a scanner connected to a PC running Microsoft Windows. Images were analyzed using Image-Pro Plus 5.0 (Media Cybernetics, Silver Spring, USA). Each cerebral area was traced with the mouse cursor control using the Paxinos and Watson [33] atlas as reference and the light transmittance was determined as the gray level. Gray level of densitometric measurements calculated after subtraction of the