Neuropharmacology 75 (2013) 223–232

Contents lists available at ScienceDirect

Neuropharmacology

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

Early postnatal inhibition of serotonin synthesis results in long-term reductions of perseverative behaviors, but not aggression, in MAO A-deficient mice

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ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form
2 July 2013
Accepted 8 July 2013

Keywords:
Serotonin
Monoamine oxidase A
Animal models
Early developmental stages
Perseverative behaviors
Aggression

ABSTRACT

Monoamine oxidase (MAO) A, the major enzyme catalyzing the oxidative degradation of serotonin (5-hydroxytryptamine, 5-HT), plays a key role in emotional regulation. In humans and mice, MAO-A deficiency results in high 5-HT levels, antisocial, aggressive, and perseverative behaviors. We previously showed that the elevation in brain 5-HT levels in MAO-A knockout (KO) mice is particularly marked during the first two weeks of postnatal life. Building on this finding, we hypothesized that the reduction of 5-HT levels during these early stages may lead to enduring attenuations of the aggression and other behavioral aberrances observed in MAO-A KO mice. To test this possibility, MAO-A KO mice were treated with daily injections of a 5-HT synthesis blocker, the tryptophan hydroxylase inhibitor p-chlorophenylalanine (pCPA, 300 mg/kg/day, IP), from postnatal day 1 through 7. As expected, this regimen significantly reduced 5-HT forebrain levels in MAO-A KO pups. These neurochemical changes persisted throughout adulthood, and resulted in significant reductions in marble-burying behavior, as well as increases in spontaneous alternations within a T-maze. Conversely, pCPA-treated MAO-A KO mice did not exhibit significant changes in anxiety-like behaviors in a novel open-field and elevated plus-maze; furthermore, this regimen did not modify their social deficits, aggressive behaviors and impairments in tactile sensitivity. Treatment with pCPA from postnatal day 8 through 14 elicited similar, yet milder, behavioral effects on marble-burying behavior. These results suggest that early developmental enhancements in 5-HT levels have long-term effects on the modulation of behavioral flexibility associated with MAO-A deficiency.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) plays a fundamental role in the orchestration of emotional reactivity and in the pathophysiology of numerous mental disorders, ranging from anxiety to pathological aggression (Baldwin and Rudge, 1995; Lucki, 1998; Olivier, 2004). One of the most important mechanisms for the regulation of 5-HT neurotransmission is its metabolism by oxidative deamination, primarily catalyzed by monoamine oxidase (MAO) A (Bortolato et al., 2008, 2009). This mitochondrial membrane-bound enzyme, which is encoded by a gene located on the X-chromosome in all vertebrate species (Bortolato et al., 2008), also serves the degradation of other monoamine transmitters, such as norepinephrine (NE) and dopamine (DA) (Shih et al., 1999; Bortolato et al., 2010). The bulk of evidence indicates that low MAO-A brain activity may be a viable marker for the severity of antisocial traits (Alia-Klein et al., 2008). In addition, several studies have documented that *MAOA* allelic variants associated with low catalytic activity predispose maltreated and abused boys to develop antisocial and aggressive traits (Caspi et al., 2002; Kim-Cohen et al., 2006; Fergusson et al., 2011).

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http://dx.doi.org/10.1016/j.neuropharm.2013.07.003

In humans, a nonsense mutation of the *MAOA* gene results in Brunner syndrome, an X-linked condition characterized by antisocial and disruptive traits, violent aggression in response to sudden stressors, repetitive behaviors and mild cognitive impairments (Brunner et al., 1993a, 1993b). Consistently, MAO-A knockout (KO) mice display marked aggression, in association with other phenotypic alterations, including social and communication deficits, perseverative and neophobic-like responses, sensory disturbances, as well as disrupted barrel fields in the somatosensory cortex (Cases et al., 1995; Godar et al., 2011; Bortolato et al., 2013a). These aberrances are accompanied by elevated 5-HT brain concentrations, particularly during the first two postnatal weeks of life (Cases et al., 1995).

Several findings suggest that the phenotypic abnormalities associated with MAO-A deficiency may depend on their high 5-HT brain levels during early postnatal stages: first, inhibition of 5-HT synthesis with the tryptophan hydroxylase inhibitor *p*-chloro-phenylalanine (pCPA) during the first postnatal weeks was shown to normalize barrel field morphogenesis (Cases et al., 1996); second, the aberrant phenotypes in MAO-A KO mice are rescued by the reinstatement of this enzyme in the forebrain at birth (Chen et al., 2007); third, perinatal administration of MAO-A blockers induces long-term aggressive responses and other behavioral changes (Whitaker-Azmitia et al., 1994; Mejia et al., 2002).

In contrast with this background, the long-term behavioral impact of early pCPA administration on MAO-A KO mice has not yet been examined. Thus, in the present study we examined whether pCPA administration in the first two postnatal weeks of life may affect aggression and other behavioral phenotypes of MAO-A KO mice, including social deficits, perseverative and alterations of anxiety-like responses. In addition, we verified whether this treatment may induce specific alterations of NMDA glutamate receptor subunit composition, in view of our recent discovery that alterations in this receptor are likely to contribute to the aggression in MAO-A KO mice (Bortolato et al., 2012).

2. Materials and methods

2.1. Subjects and treatment schedule

We used experimentally naïve male 129S6 MAO-A^{A863T} KO and wild-type (WT) mice, generated and genotyped as previously described (Scott et al., 2008). Since MAO A is located on the X-chromosome, mice were bred from WT males crossed with heterozygous MAO-A KO females and litters were culled to 6–8 pups. Animals were injected with either pCPA (300 mg/kg/day, i.p., q.d.; Sigma-Aldrich, St. Louis, MO, USA) or saline solution during the first or the second postnatal week (Fig.1). Intraperitoneal injections were performed at an injection volume of 10 ml/kg, using an injector attached to a micro-syringe. In addition, a group of mice was left untreated to control for the potentially stressful influence of early daily injections. In preliminary studies, we observed that the stress associated with early tattooing or permanent marking led to alterations of maternal behavior (including cannibalism) and/or enduring alterations of stress response. To avoid these potential confounds, all male offspring of each mother (both WT and MAO-A KO littermates) was subjected to the same treatment conditions. Litter effects were minimized by using animals from at least 8 litters for each treatment group for P1–P7 injections and 4 litters for each treatment group for P8–P14 injections. No more than 2 homogenotypic male littermates (randomly selected) in each group were used. Once weaned, male mice were group-housed with other conspecifics from other litters and treatment groups, and received *ad libitum* access to food and water, in facilities maintained at 22 °C on a 12 h: 12 h light/dark cycle.

Mice were isolated on postnatal day 80 (P80) and tested between P90 and P120 (adulthood). This isolation regimen was selected to minimize the potentially detrimental behavioral outcomes of aggressive interactions with MAO-A KO male mice (which display spontaneous aggression towards cage mates); in addition, these conditions are optimal to elicit mild levels of aggressiveness in WT males during the resident-intruder test.

All *in vivo* experimental procedures were in compliance with the National Institute of Health guidelines and approved by the Animal Use Committees of the University of Southern California and University of Cagliari.

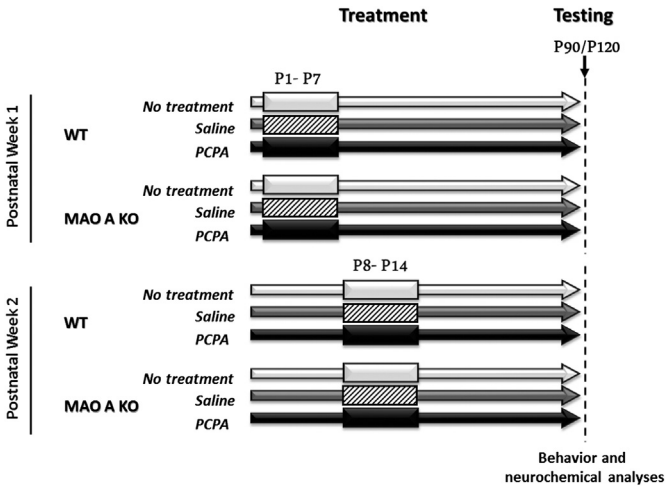

Fig. 1. Schematic representation of the experimental design for pCPA administration during the first two postnatal weeks. Behavioral and neurochemical analyses were performed between postnatal day 90 and postnatal day 120 (corresponding to adulthood).

2.2. Neurochemical analyses

For biochemical analyses, male mice were deeply anesthetized with isoflurane and promptly decapitated. Brains were removed within approximately 30 s after decapitation, and forebrain regions were freshly dissected as described by Spijker (2011). Prefrontal cortices were obtained from adult brains by coronal sections (Spijker, 2011). Whole pup forebrains were harvested, to obtain sufficient biological material for further analyses.

2.2.1. HPLC determination of monoamine levels

Forebrain samples were kept in dry ice and rapidly homogenized with an ultrasonic tissue disrupter (Sonoplus HD60, Bandelin, Germany), in a solution containing 0.1 M trichloroacetic acid, 10 mM sodium acetate, and 0.1 mM EDTA; 1 μM isoproterenol was used as an internal standard. The homogenates were centrifuged, and the supernatants were used for high performance liquid chromatography (HPLC) analysis. The mobile phase was the same as the homogenization buffer with 7% methanol for detection of 5-HT. The mobile phase was filtered and deaerated, and the pump speed (Shimadzu LC-6A liquid chromatograph, Columbia, MD, USA) was 1.5 ml/min. The reverse-phase column used was a Rexchrom S50100-ODS C18 column with a length of 25 cm and an internal diameter of 4.6 mm. The compounds were measured at +0.7 V using a Shimadzu L-ECD-6A electrochemical detector.

2.2.2. Western blot analyses of NMDA receptor subunits

Prefrontal cortex samples were homogenized in ice cold buffer (50 mM HEPES, pH 7.4, 40 mM β-glycerol phosphate, 1 mM sodium orthovanadate, 50 mM Sodium Fluoride, 2 mM EDTA, 20 μM $ZnCl_2$, 1% Sodium deoxycholate) and protease inhibitors. The homogenates were centrifuged at 75,000 × *g* for 30 min at 4 °C and supernatants used for western blotting assays. Equal amounts of protein from each sample (20 μg) were used in standard western-blot assays for NR1 (Cell Signaling, Danvers, MA, USA), NR2B and NR2A (NeuroMab, Davis, CA, USA). Blots were developed by chemiluminescence detection in a Kodak 4000 MM imaging station. Bands were quantified in arbitrary units and normalized for protein concentrations using β-actin as loading control.

2.3. Behavioral testing

Environmental light and sound levels were maintained at 10 lux and 70 dB for all behavioral tests unless otherwise indicated. Testing was performed between 10 am and 3 pm with lights on at 6 am in the listed sequence below. Experiments on animals injected during the first and second postnatal weeks were performed and analyzed separately. All behaviors were video-recorded and analyzed by trained observers, who were blind to the genotype and treatment of the mice. To reduce carryover effects due to stress ensuing repeated testing, each animal was used for no more than 3 different tests (with a time interval of at least 1 week between two consecutive tests) and the order of testing was randomized (with the exception of social interaction and resident-intruder aggression tests, which were always performed at the end of behavioral batteries).

2.3.1. Open field

Analysis of open-field behaviors was performed to assess locomotor and exploratory responses, as well as anxiety-related reactivity. Testing was performed