Physiology & Behavior 124 (2014) 37–44

Contents lists available at ScienceDirect

Physiology & Behavior

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

Altered expression of 5-HT1A receptors in adult rats induced by neonatal treatment with clomipramine

Ofelia Limón-Morales [a], Cesar Soria-Fregozo [b], Marcela Arteaga-Silva [c], Gonzalo Vázquez-Palacios [d], Herlinda Bonilla-Jaime [c,*]

[a] Posgrado en Biologia Experimental, Universidad Autonoma Metropolitana-Iztapalapa Apartado, Postal 55 535, C.P 09340 D.F. México, Mexico
[b] Laboratorio de Psicobiologia, Centro Universitario de los Lagos, Universidad de Guadalajara, Mexico
[c] Departamento de Biología de la Reproduccion, Universidad Autonoma Metropolitana-Iztapalapa Apartado, Postal 55 535, C.P 09340 D.F. México, Mexico
[d] Colegio de Ciencia y Tecnología, Universidad Autónoma de la Ciudad de México-San Lorenzo Tezonco, Av. Prolongación San Isidro 151, Col. San Lorenzo Tezonco, Deleg, Iztapalapa CP 09790, Mexico

ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form 14 December 2012
Accepted 22 October 2013

Keywords:
5-HT1A receptor
Clomipramine
Depression
Forced swimming test
8-OH-DPAT
PCPA
RT-PCR

ABSTRACT

Chronic administration of clomipramine (CMI) to neonatal rats produces behaviors that resemble a depressive state in adulthood. Dysfunctions in the activity of the central nervous system's serotonergic function are important in understanding the pathophysiology of depression. The serotonin system is implicated in major depression and suicide and is negatively regulated by somatodendritic 5-HT1A autoreceptors. Desensitization of 5-HT1A autoreceptors is implicated in the long latency of some antidepressant treatments. Alterations in 5-HT1A receptor levels are reported in depression and suicide. In this study, we analyzed the effect of neonatal administration of CMI on the activity of 5-HT1A receptors, both pre- and post-synaptically, by administering an agonist of 5-HT1A receptors, 8-OH-DPAT, and then subjecting the rats to the forced swimming test (FST) a common procedure used to detect signs of depression in rats. Also measured were levels of the mRNA expression of 5-HT1A receptors in the dorsal raphe (DR), the hypothalamus and the hippocampus. Wistar rats were injected twice daily with CMI at doses of 15 mg kg^{-1} or saline as vehicle (CON) via s.c. from postnatal day 8 for 14 days. At 3–4 months of age, one set of rats from each group (CON, CMI) was evaluated for the effect of a selective agonist to the 5-HT1A receptor subtype, 8-OH-DPAT, by testing in the FST. Also determined was the participation of the pre- or postsynaptic 5-HT1A receptor in the antidepressant-like action of 8-OH-DPAT. This involved administering an inhibitor of tryptophan hydroxylase, parachlorophenylalanine (PCPA), and pretreatment with 8-OH-DPAT before the FST test and to evaluate the rectal temperature and locomotor activity. The expression of the mRNA of the 5-HT1A receptors was examined in the dorsal raphe nucleus, the hypothalamus and the hippocampus using the semiquantitative RT-PCR method. The results from this study corroborate that neonatal treatment with clomipramine induces a pronounced immobility in the FST when animals reach adulthood, manifested by a significant decrease in swimming behavior, though counts of climbing behavior were not modified. This effect was similar in magnitude when 8-OH-DPAT was administered to CON group. Furthermore, the administration of 8-OH-DPAT induces a significant and similar increase in rectal temperature and locomotor activity in both the CON as in the CMI group. Neonatal treatment with CMI resulted in a significant decrease in the expression of the mRNA of the 5-HT1A receptors in the DR (% more than vehicle) in adulthood. In the case of the postsynaptic receptors located in the hypothalamus and hippocampus, neonatal treatment with CMI induced a significant increase in the mRNA expression of the 5-HT1A receptors. These data suggest that neonatal treatment with CMI induces a downregulation of the mRNA of the 5-HT1A autoreceptors in the DR, and an increment in the expression of the postsynaptic 5-HT1A receptors. The results after the administration of PCPA and 8-OH-DPAT on FST, rectal temperature and locomotor activity for both groups suggest that the function of postsynaptic receptors remains unchanged. All together these data show that the depressive behavior observed in adulthood in this animal model may be associated with long-term alterations in the expression of the mRNA of the 5-HT1A receptors.

1. Introduction

It is currently estimated that 1 in 5 people will develop a major depressive episode at least once in their lifetime, making this one of the most recurrent and disabling diseases worldwide [1]. The occurrence

* Corresponding author at: Departamento de Biologia de la Reproduccion, Universidad Autonoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco No. 186, Col. Vicentina, C.P. 09340 Iztapalapa, D.F. Mexico, Mexico. Tel.: +52 55 5804 4701; fax: +52 55 5804 4930.
E-mail address: bjh@xanum.uam.mx (H. Bonilla-Jaime).

0031-9384/$ – see front matter.
http://dx.doi.org/10.1016/j.physbeh.2013.10.026

of this condition has been strongly linked to changes in the function of the serotonergic system [2]. Especially, alterations in the 5-HT1A receptor functions and levels have been implicated in mood disorders. In particular, 5-HT1A receptors negatively regulate the activity of 5-HT neurons, and are expressed as both a presynaptic autoreceptor on raphe neurons and as a major postsynaptic receptor in the hippocampal, cortical and hypothalamic regions involved in mood, emotion and stress responses [3,4]. A general hypothesis regarding 5-HT in depression suggests that 5-HT1A receptors may be downregulated, which can lead to a negligible occupation of the receptor by 5-HT to produce an appropriate physiological response [2,5,6]. It has also been shown that desensitization participates in the antidepressant effect of selective inhibitors of serotonin reuptake (SSRIs), such as fluoxetine [5,6]. In addition, it has been determined that both the number and expression of mRNA for 5-HT_{1A} receptors are altered in the ventrolateral nucleus of the hypothalamus [7], the dorsal raphe nuclei [8,9], the frontal cortex, and the amygdala [3,10–12] of suicide victims, as well as in animal models of depression using Flinders rats [10,13].

One method commonly used to produce rats with behavioral changes consistent with human depression is the neonatal administration of clomipramine (CMI), a monoamine re-uptake inhibitor [14]. Upon reaching adulthood, rats that received this treatment exhibit behavioral abnormalities that resemble endogenous depression [14], including reduced aggressiveness [15], decreased pleasure-seeking behaviors [16], diminished sexual activity [17,18], shorter REM sleep onset, and more REM sleep periods [19,16]. Some of the behavioral abnormalities in these rats (sexual, aggressive and motor) begin to normalize after treatment with antidepressants, such as imipramine, or after REM sleep deprivation [14]. It has been reported that neonatal CMI treatment results in a pronounced immobility in the FST, compared to controls that receive only saline neonatal treatment [17,20]. In addition, it has been reported that CMI-treated rats show neurochemical alterations in the serotonergic system [21–23], such as a decrease in the concentration of 5-HT in the frontal cortex, hippocampus, brainstem, hypothalamus and septum [15], as well as a decrease in neuronal firing rates in the dorsal raphe (DR) [24], all of which could suggest alterations in the expression or activity of the 5-HT1A receptors in the structures involved in depression. The FST is an extensively used model in which a behavioral change is induced by acute stress [25,26]. As a model, it has shown predictive validity [25,28], pharmacological selectivity [27], and construct- [28] and face-validity [25,26]. In this test, rats are placed in water such that they are forced to swim. After a single pretest session, most of the subjects showed increased immobility when retested for swimming 24 h later; i.e., they showed despair reflected as immobility, which was defined as floating without struggling while making only those movements necessary to keep the head above water. Increases or decreases in immobility time are interpreted as antidepressive or depression-like actions, respectively [25]. In addition to immobility, rats may show active behaviors that reflect their interest in attempting to avoid this adverse condition. Those behaviors have been divided into two categories: swimming and climbing (vide infra). The modified version of this model [29] makes it possible to infer the participation of different neurotransmitters in the effects of antidepressant drugs. Thus, a decrease in immobility accompanied by an increase in swimming denotes activation of the serotoninergic system, while an increase in climbing indicates activation of the catecholaminergic systems [30]. On another point, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a selective agonist 5-HT1A receptor, has been shown to exercise antidepressant-like activity in animal models of depression such as the FST. The effect of chronic treatment with 8-OH-DPAT on rats' behavior in the FST has been studied in animals injected intracerebroventricularly with 5,7-dihydroxytryptamine (5,7-DHT) or parachlorophenylalanine (PCPA), showing that integrity of the serotonergic system is required [31–33], as some findings indicate that the microinjection of 8-OH-DPAT directly into the DR may produce antidepressant effects [31]. Moreover, the destruction of serotonergic neurons in the DR cancels the antidepressant effect while also blocking serotonin synthesis (5-HT) [32]. PCPA, meanwhile, blocks the antidepressant effect of 8-OH-DPAT in the FST [33].

The aim of this study was to investigate whether potential changes in the function and levels of the 5-HT1A receptors are implicated in the behavioral alterations induced by neonatal treatment with CMI by administering an agonist of 5-HT1A receptors [34,35], 8-OH-DPAT, before evaluating performance of the FST [25,31], ascertaining motor activity and rectal temperature [36], and also measuring levels of the expression of the mRNA of 5-HT1A receptors in the DR, hypothalamus and hippocampus. An alteration in the function and levels of the 5-HT1A receptor has been implicated in mood disorders. In addition, it has been observed that the expression of mRNA for pre- and postsynaptic 5-HT1A receptors is altered in suicide victims. We hypothesized that neonatal treatment with CMI would downregulate the 5-HT1A receptor of the DR nucleus and induce an alteration in the function of the 5-HT1A receptor.

2. Materials and methods

2.1. Neonatal treatment

The rats used were obtained from our own vivarium. All experiments were carried out in strict accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) [37]. A group of 21 pregnant Wistar rats was used to obtain the different litters. On postnatal day 3, the male pups were randomly cross-fostered to maintain a uniform number of pups in each litter ($n = 6$ pups/mother). The female pups were eliminated from this study. The animals were housed according to treatment (control or experimental), but all were kept on a 12-h light–dark cycle (lights on at 9:00, off at 21:00 h) with ad libitum access to food and water. From neonatal days 8 to 21, the experimental group (CMI) received subcutaneous injections of clomipramine (15 mg/kg body weight, in 0.1 ml saline solution), while the male control pups (CON) received subcutaneous injections of the vehicle only (0.1 ml of saline solution). Pups were injected twice a day (9:00 am, 18:00 pm) while still with the mother rats. At 23 days of age, the pups were weaned, housed in groups of 10, and maintained under standard conditions for the ensuing 3 months. At the age of 3 months, independent groups of both the CMI-treated and CON rats were subjected to the following experiments: A) Determination of the RT-PCR of the 5-HT1A receptor (2 groups: neonatal CON and neonatal CMI) and; B) Pharmacological studies to evaluate the function of the 5-HT1A receptor using the FST, rectal temperature and motor activity.

2.2. Pharmacological studies

The first part of the experiment was designed to evaluate the effect of a selective agonist to the 5-HT1A receptor subtype, 8-OH-DPAT. Independent groups ($n = 7$) of CON and CMI rats were administered one of the following doses of 8-OH-DPAT: saline, 0.25 or 0.5 mg/kg. 8-OH-DPAT was administered 48 h, 24 h, and 30 min before the 5-min FST test session. The second part of the experiment sought to determine the participation of the pre- or post-synaptic 5-HT1A receptors in the antidepressant-like action of 8-OH-DPAT (0.5 mg/kg). In order to test this, an inhibitor of tryptophan hydroxylase, parachlorophenylalanine (PCPA), was administered to both groups of animals (CON and CMI). PCPA was given at a dose of 100 mg/kg following a sub-chronic treatment schedule at 72, 48 and 24 h before the 5-min FST test session. The onset of administration of 8-OH-DPAT was on the second day of treatment with PCPA (48 h). The 8-OH-DPAT was administered 30 min after the PCPA. The animals received three injections of 8-OH-DPAT following the 48 h, 24 h, and 30 min schedule, before the 5-min FST test session. All experiments were conducted using an independent group design. In another experiment, two groups of animals (CON, CMI) were administered a dose of 8-OH-DPAT (0.25 or 0.5 mg/kg) or saline as controls