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Effects of n-3 PUFA enriched and n-3 PUFA deficient diets in naïve and Aβ-treated female rats

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ABSTRACT

Depression is one of the most common psychiatric diseases and the prevalence of depressive symptoms in women is almost twice compared to men, although the reasons of this gender difference are not fully understood yet. Recently, soluble $A\beta_{1-42}$ peptide has been receiving great importance in the development of depression, also since depression is highly comorbid with Alzheimer's disease and other neurodegenerative illnesses. Accordingly, we have previously shown that central Aβ injection is able to elicit depressive-like phenotype in male rats. In the present study, we reproduced for the first time the Aβ-induced depressive-like model in female rats, evaluating behavioural and neurochemical outcomes. Moreover, we studied the effect of lifelong exposure to either n-3 PUFA enriched or n-3 PUFA deficient diet, in female rats, both intact and after central Aβ administration. Our results confirmed the Aβ-induced depressive-like profile also in female rats. Moreover, chronic exposure to n-3 PUFA deficient diet led to highly negative alterations in behavioural and neurochemical parameters, while lifelong exposure to n-3 PUFA enriched diet was able to restore the Aβ-induced depressive-like profile in female rats. In conclusion, the Aβ-induced depressive-like profile was reversed by n-3 PUFA supplementation, indicating a possible therapeutic role of n-3 PUFA in the treatment of the burden of depressive disorders.

1. Introduction

Depression is one of the most common psychiatric diseases and the prevalence of depressive symptoms has reached epidemic proportions during the last few decades [32]. In this regard, several studies reported that depression is more prevalent in women compared to men [32,40,50]. Although reasons of this gender difference are not fully understood yet, women show different response to sex hormones, that might ultimately influence behaviour and brain functions [51]. In particular, estrogens modulate several neural and behavioural functions, including mood, cognitive function, blood pressure regulation, motor coordination, pain, and opioid sensitivity [52]. In addition, it has been shown that estrogens also affect neurotrophic functions and monoamine neurotransmission in several brain areas, thus they might ultimately be involved in the pathogenesis of depressive-like disorders [9]. Such evidence suggests that the antidepressant therapy should be personalized, taking into account also gender differences [82,90]. In addition, a series of studies indicated that estrogens modulate the metabolic production of different endogenous and exogenous molecules [6,43,58]. Among these molecules, it has been reported that estrogens stimulate the conversion of essential fatty acids into their longer chain metabolites, such as α-linolenic acid conversion into docosahexanoic acid (DHA) [11,31]. DHA is a key n-3 polyunsaturated fatty acid (PUFA) involved in the Central Nervous System (CNS) development [16] and, thus, it is fundamental during pregnancy and early stage of childhood [24]. DHA and arachidonic acid (AA, 20:4n-6) are biologically important PUFAs, and can be supplied either directly from diet or by metabolic conversion of their essential precursors α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), respectively [64]. DHA, AA and their mediators modulate several processes, such as signal pathways, membrane fluidity, neurotransmission, neuroinflammation and cell survival [24]. During embryonic life and lactation, PUFAs intake exclusively depends on maternal diet, as the metabolic conversion of essential precursors cannot be accomplished [42]. Indeed, *in utero*

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exposure to unbalanced diet can be an important risk factor for mental disorders in later adulthood [42]. Modern western diets are characterized by low fish consumption and more junk food, resulting in n-3 PUFA deficiency and abnormal n-6 PUFA increase, respectively [81]. This unbalanced n-6/n-3 ratio is considered to be detrimental for the CNS functioning. Indeed, recent research suggests an etiological role for n-3 PUFA deficiency in mood disorders, such as Major Depressive Disorder (MDD) [34,55]. Accordingly, different epidemiological studies reported an inverse correlation between n-3 PUFA intake and depressive symptoms among women in United States [7,8]. We have previously shown that lifelong deficiency of n-3 PUFA leads to a depressive-like phenotype associated with reduced serotonin (5-HT) levels and increased soluble amyloid beta $(A\beta)_{1-42}$ concentrations [64] in male rats. The $A\beta_{1-42}$ peptide and its oligomeric forms have been demonstrated to have powerful neurotoxic effects [73,79]. Recently, soluble $A\beta_{1-42}$ peptide has been received great importance in the development of depression, also since depression is highly comorbid with Alzheimer's disease and other neurodegenerative illnesses [72,73,89]. In this regard, we have previously shown that the injection of a solution of soluble $A\beta_{1-42}$ in the ventricular area of male rats can evoke a depressive-like phenotype associated with reduced cortical 5-HT and neurotrophins, such as Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF) [15,78].

In order to avoid the variability that female hormonal cycle could induce [3], the majority of animal studies on depression use males. However, the US National Institute of Health is strongly encouraging preclinical research on females [40]. Hence, considering also the higher incidence of depressive disorders in women, the development of preclinical models of depressive-like profile in females is becoming necessary [18].

In the present study, we evaluated the effect of lifelong exposure to either n-3 PUFA enriched or n-3 PUFA deficient diet in female rats exposed to $A\beta_{1-42}$ administration.

2. Methods

2.1. Animals

Adult (250–300 g) Wistar rats (Harlan, S. Pietro al Natisone, Udine) were used in this study. They were housed at constant room temperature (22 ± 1 °C) and relative humidity (55 ± 5%) under a 12 h light/dark cycle (lights on at the 7 A.M.) with *ad libitum* access to food and water.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines of the Italian Ministry of Health (D.L. 26/2014), the Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2004), the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. All procedures involving animals were approved by the Italian Ministry of Health (protocol number: B2EF8.17) and were conducted in accordance to ARRIVE guidelines. Animal welfare was daily monitored through the entire period of experimental procedures. No signs of distress were evidenced, anyway all efforts were made to minimize the number of animals used and their suffering.

2.2. Diets

One male and two female rats were housed together for mating. Animals were exposed to specific diets mimicking lifelong n-3 PUFA deficiency or supplementation, as previously described [2,42,64]. After mating, dams were randomly assigned to the group fed with a diet (laboratorio dottori Piccioni srl, Gessate, Milan Italy) containing 6% total fat in the form of only rapeseed oil (n-3 enriched, rich in α linolenic acid 18:3n-3) or peanut oil (n-3 deficient, rich in linoleic acid 18:2n-6) throughout gestation and lactation. As control group, dams were fed with a diet containing 6% total fat in the form of 3% of peanut oil plus 3% of rapeseed oil, called control diet. After weaning, offspring continued to be subjected to the same diet throughout life. All experiments were carried out in female eight-week-old rats. Estrus cycle synchronization was determined by vaginal smear and confirmed by serum estradiol concentration. However, considering that pro-estrous/estrus events under normal lighting schedules (as in the present study) tend to occur during the late afternoon to early hours of the morning [96] procedures involving alive animals were performed in the morning (09.00–12.00 h).

2.3. Aβ administration

The $A\beta_{1-42}$ peptide was purchased from Tocris (Bristol, UK) and was freshly prepared in sterile double-distilled water (vehicle) at a concentration of 4 μM as previously described [15]. Seven-weeks-old rats were anaesthetized with 3.6 ml/kg Equithesin intraperitoneally (i.p.; composition: 1.2 g sodium pentobarbital; 5.3 g chloral hydrate; 2.7 g MgSO4; 49.5 ml propylene glycol; 12.5 ml ethanol and 58 ml distilled water) (Sigma Aldrich. Milan Italy) and secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Coordinates from bregma for intracerebroventricular (icv) infusions were based on the atlas of Paxinos and Watson [69]: AP = −0.5, ML = +1.2 and DV = - 3.2, (incisor bar at -3.3 mm). Soluble Aβ (5 μl) was delivered through a 25 μl Hamilton microsyringe at 2 μl/min infusion rate over a period of 2.5 min. Infusion needle was left in place for additional 5 min in order to avoid elapsing during removal. Control rats received vehicle only, because outcomes observed from preliminary used reverse $A\beta_{42-1}$ were indistinguishable from vehicle alone (unpublished observations). The needle track placement of was verified at the time of dissection. All experimental procedures were carried out 7 days after icv administration (SHAM or Aβ-treated groups).

2.4. Forced swimming test

The forced swimming test (FST) is a reliable task for discriminating depressive state in animals and is widely used for predicting antidepressant properties of drugs [74]. On the first of the two test days, animals were placed individually in inescapable Perspex cylinders (diameter 23 cm; height 70 cm) filled with water at constant temperature of 25 ± 1 °C at 30 cm of height [17].

During the preconditioning period, animals were videotaped for 15 min, then were returned to their home cages after drying. Twenty-four h later, each rat was positioned in the water-filled cylinder for 5 min and video-recorded. Frequencies of the following behaviors were scored by a blind observer: struggling (tentative of escaping), swimming (moving around the cylinder) and immobility (remaining afloat making only the necessary movements to keep its head above the water). Data were expressed as frequency on 5 s counts.

2.5. Post-mortem tissue analysis

Brains were immediately removed from euthanized rats, and cooled on ice for dissection of prefrontal cortex (PFC) and hypothalamic areas, according to the atlas of Paxinos and Watson [69]. Tissues were frozen and stored at −80 °C until analyses were carried out.

2.6. High-performance liquid chromatography (HPLC) quantifications

5-HT, 5-hydroxyindolacetic acid (5-HIAA) and dopamine (DA) concentrations were determined by HPLC coupled with an electrochemical detector (Ultimate ECD, Dionex Scientific, Milan, Italy). Separation was accomplished by a LC18 reverse phase column (Kinetex, 150 mm × 4.2 mm, ODS 5 μm; Phenomenex, Castel Maggiore-Bologna, Italy) and detection was performed through a thin-layer amperometric cell (Dionex, ThermoScientifics, Milan, Italy) with a 5 mm diameter