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Gene expression profile of estrogen receptors alpha and beta in rat brain during aging and following high fat diet

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ABSTRACT

The "sex-hormone" estrogen-17β promotes several cognitive functions and is a master regulator of brain bioenergetics via the estrogen receptors α and β (*ERα* and *ERβ*). In this work, by using Real-Time PCR analysis, we evaluated the effect of aging and high fat diet (HFD) on *ERα* and *ERβ* expression in rat hippocampus and cortex. In young rats, *ERβ* is abundant in cortex and *ERα* in hippocampus. During the aging, in the cortex, we observe a general decrease in *ERα* and *ERβ* expression; in hippocampus *ERα* increases and *ERβ* decreases. *ER* expression patterns in rat brain are also affected by the administration of an HFD. In cortex, after 4 weeks of HFD, *ERβ* transcripts are down-regulated, whereas *ERα* levels remain unchanged; after 12 weeks, both ERα and *ERβ* expression is up-regulated. In the hippocampus, the level of *ERβ* transcripts does not change following HDF, whereas *ERα* expression is affected by HDF, in a time-dependent manner: it increases after the 4-week treatment and decreases after 12 weeks. Possible involvements of these receptors in the control of cortex and hippocampus functions during aging and in the modulation of energetic metabolism and feeding behaviour are discussed.

1. Introduction

Estrogens are sex hormones involved in the development and maintenance of normal reproductive functions [1–3]. These hormones exert their biological effects through the interaction of two primary estrogen receptors, members of the nuclear steroid receptors superfamily: estrogen receptor-alpha (ERα), and estrogen receptor-beta (ERβ).

17β-Estradiol (E2) is the most potent and predominant form of estrogen; besides its recognized role in reproduction, it shows a number of effects on cognition and brain function [4–7]. In different brain regions, aromatase activity catalyzes E2 synthesis from androgen precursors and/or cholesterol. E2 is neurotrophic and neuroprotective and some recent results, although controversial, suggest that E2 synthesized within hippocampus and prefrontal cortex may contribute to the normal processes of memory consolidation [8–11]. The effects of E2 on several brain processes decline with age, including the ability to preserve the blood–brain barrier and to protect from stroke and neuroinflammation [12,13].

Accordingly with E2 functions, both *ERα* and *ERβ* subtypes are widely distributed in the brain regions, in particular in the hippocampus, the frontal cortex, and the amygdala [4,14]. The alteration in the normal distribution of ERs has been observed in hippocampal neurons of Alzheimer's disease (AD) patients [15].

It has been demonstrated that the two ER subtypes play different roles in E2-regulated gene expression and neuronal physiology [16]; the differential activation of *ERα* or *ERβ* is able to influence the expression of neuroprotective genes [17,18].

Data on the differential expression of *ER* genes in the central nervous system (CNS), mostly achieved in females

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and collected in the rat, the mouse or the human, are often contradictory [19–21]. In the rat hippocampus, *ERα* is transcriptionally more active than *ERβ* [7], and a significant decrease in the number of neurons positive to estrogen receptors during aging is observed [22]. In addition, *ER*s seem to have a gender specificity; indeed, *ERβ* expression in the female hippocampus is greater than in the male one [23]. In the cerebral cortex of the same animals, the limited data available show *ERβ* as the predominant receptor subtype immunolocalized [24].

In mouse, *ERα* is the predominant subtype in the hippocampus and in most of hypothalamus, whereas in the cerebral cortex *ERβ* is the main receptor subtype observed [25]. Conversely, in human and primates *ERβ* is the primary receptor reported in hippocampus [26,27]. In humans, it has been found that following the neurological diseases influencing memory, such as AD, the expression of *ERα* decreases while *ERβ* increases [28–31].

The relationship between E2 and ERs in the brain involves the regulation of food intake. In women, the daily food intake changes in relation to the E2 secretion during the menstrual cycle: during the follicular phase, the daily food intake is lower in respect to the luteal phase [32,33]. Also in rat, the increase in plasma E2 concentration during the preovulatory phase is associated with a transient decrease in food intake [34]. In the CNS, both *ERα* and *ERβ* are expressed in areas associated with satiety and feeding, such as hypothalamus and pituitary [35]; it has been also demonstrated that ER activation is involved in the metabolic effect of insulin signaling in the brain [36]. Finally, E2 has been proposed to act directly and indirectly to decrease orexigenic or anorexigenic peptides and food intake: for instance, *ERα* null mice are obese, insulin resistant, and show decreased energy expenditure [35,37].

In this study, by using Real-Time PCR analysis, we investigated the expression pattern of *ERα* and *ERβ* genes during aging and after a high fat diet (HFD) in rat brain. In particular, we evaluated the *ERα* and *ERβ* expression profiles in the cerebral cortex and hippocampus of pubertal young (2-months), adult (4- and 8-month), and middle-aged (16-month) rats. Our results demonstrate that *ERα* and *ERβ* subtypes have specific tissue- and age-related expression patterns that are unbalanced by an HFD that leads to the obese phenotype.

2. Materials and methods

2.1. Animal and experimental design

Male Wistar rats (Charles River, Calco, Como, Italy) of 2 months of age (adolescent), with the same starting body weight (160 ± 10 g), were individually caged in a temperature-controlled room (23 ± 1 °C) with a 12 h light/12-h dark cycle. Animals were housed in the Animal Care Facility at the Department of Biology, with *ad libitum* access to water and to a standard diet (SD, 10.6% fat J/J, 60,4% carbohydrate, 15.47 KJ/g) (Mucedola 4RF21; Settimo Milanese, Milan, Italy) up to 4 (social maturity, group 1), 8 (adulthood, group 2), or 16 (middle-aged, group 3) months. Six animals for each group were anesthetized with chloral hydrate (40 mg/100 g body wt) and killed by decapitation. Group 4 was made of eight adolescent rats sacrificed four days after their arrival in the animal facility.

In parallel, another group of rats (referred to as HFD, $n = 12$) received a high-fat diet rich in lard (40% fat J/J, 31% carbohydrate, 19.23 KJ/g). The HFD was formulated to differ from the standard diet in the fat and carbohydrate contribution to the energy value but to be identical in terms of proteins, vitamins, minerals and fibers [38]. Six animals of both SD and HFD groups were sacrificed after 4 weeks and other six individuals after 12 weeks. Throughout the experimental period, body weights and food intakes were monitored daily to calculate the body-weight gain. Spilled food was collected and compensated in readjusting the calculation of food intake.

At the end of the experimental period, animals of each group were anesthetized by chloral hydrate (40 mg/100 g body wt) and killed by decapitation. The brains were quickly removed and the cerebral cortex and hippocampus were dissected on ice. Samples of each brain region were snap frozen in liquid nitrogen immediately and stored at −80 °C for subsequent RNA isolation. The study was performed in strict accordance with the criteria established by the National Institutes of Health. The protocol for animal care and use was approved by the Committee on the Ethics of Animal Experiments of the University of Naples Federico II.

2.2. RNA purification and cDNA synthesis

Total RNA was extracted according to the TRI-Reagent (Sigma Aldrich) protocol. The quality of each total RNA was checked by electrophoresis on 2% agarose gel stained with ethidium bromide and measuring the optical density at 260/280 nm. A ratio of 1.8–2.0 was accepted for further reverse transcription. The QuantiTect Reverse Transcription Kit (Qiagen) was used for the removal of genomic DNA contamination and for the subsequent cDNA synthesis. Approximately 1 μg of total RNA was used, according to the kit's protocol.

2.3. Quantitative Real-Time PCR analysis

The Real-Time PCR reactions were carried out in triplicate for each sample in an Applied Biosystems 7500 Real-Time System by using the Power SYBR Green Master Mix PCR (Applied Biosystems) following the procedures recommended by the manufacturer [39]. Each SYBR Green reaction (20-μl total volume) contained 12 μL of Real-Time PCR Master Mix, 1 μL of each of the forward and reverse primer (10 μM), 2 μL of cDNA diluted 1:1 and 4 μL of nuclease free water. For internal standard control, the expression of the β-actin gene was quantified [40]. A single pair of specific primers for both *ERα* and *ERβ* isoforms was designed on the nucleotide sequences of *Rattus norvegicus ERα* (NM012689.1) and *ERβ* (AF042058.1); *β-actin* primers were designed on the *R. norvegicus* template (NM031144.2). Primer sequences are reported in Table 1. The lengths of the obtained cDNA fragments were: *ERα*, 216 bp; *ERβ*, 262 bp; *β-actin*, 128 bp.

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