available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Aromatase expression in the normal and epileptic human hippocampus

Josue G. Yague[a,b], Iñigo Azcoitia[c], Javier DeFelipe[a], Luis M. Garcia-Segura[a,*], Alberto Muñoz[a,c]

[a]Instituto Cajal, CSIC, Avenida Doctor Arce 37, E-28002 Madrid, Spain
[b]Hospital Nacional de Parapléjicos, SESCAM, E-45071 Toledo, Spain
[c]Departamento de Biología Celular, Facultad de Biología, Universidad Complutense, E-28040 Madrid, Spain

ARTICLE INFO

Article history:
Accepted 28 September 2009
Available online 6 October 2009

Keywords:
Aromatase
Estradiol
Hippocampal sclerosis
Gliosis
Interneurons
Epilepsy

ABSTRACT

Aromatase is a key enzyme in estrogen biosynthesis that is involved in neuronal plasticity in the rodent hippocampus. Although aromatase mRNA expression has been detected in the human hippocampus, its cellular distribution has yet to be determined. Here, we have examined the immunohistochemical distribution of aromatase in the normal and the epileptic and sclerotic human hippocampus. In both the normal and epileptic hippocampus, aromatase was detected in numerous CA1–CA3 pyramidal neurons, in granule cells of the dentate gyrus and in interneurons that co-expressed the calcium-binding proteins calbindin, calretinin or parvalbumin. However, only a small subpopulation of astrocytes was immunoreactive for aromatase in either the normal and epileptic hippocampus. The widespread expression of aromatase in a large population of neurons in the normal and damaged hippocampus suggests that local estrogen formation may play an important role in human hippocampal function.

1. Introduction

Aromatase is a key enzyme in estrogen biosynthesis that catalyzes the conversion of testosterone to estradiol, of androstenedione to estrone, and of 16-hydroxylated dehydroepiandrostenedione to estriol (Stoffel-Wagner, 2001). Since pioneering work demonstrated that aromatase activity exists in brain tissue where it can generate estrogens from androgen precursors (Naftolin et al., 1971), the expression and distribution of aromatase in the central nervous system of several vertebrate species has been described (Ryan et al., 1972; Flores et al., 1973; Naftolin et al., 1975; Roselli et al., 1985; Schumacher and Balthazart, 1987; Jakab et al., 1993; Shinoda et al., 1994).

The hippocampal formation is involved in learning and memory processes and it is known to be a target for estrogens produced both in the gonads and locally. Accordingly, estradiol induces morphological and functional changes in hippocampal circuits (Gould et al., 1990; Woolley, 1998; Foy et al., 1999; Adams et al., 2001; Hao et al., 2003; McEwen and Milner, 2007; Hajszan et al., 2007; Spencer et al., 2008), regulates adult hippocampal neurogenesis (Ormerod and Galea, 2001; Galea et al., 2006; Suzuki et al., 2007; Pawluski et al., 2009) and exerts neuroprotective actions in the hippocampus (Azcoitia et al., 1998, Velísková et al., 2000; Garcia-Segura et al., 2001; Kuroki et al., 2001; Saravia et al., 2006). Both local estradiol production and aromatase expression have been reported in the hippocampus of different bird species, such as the zebra finch (Shen

* *Corresponding author*. Fax: +34 915854754.
E-mail address: lmgs@cajal.csic.es (L.M. Garcia-Segura).

Abbreviations: CA, cornu ammonis; CB, calbindin; CR, calretinin; DG, dentate gyrus; GFAP, glial fibrillary acidic protein; NeuN, neuron-specific nuclear protein; PV, parvalbumin; PB, phosphate buffer; PBT, phosphate buffer with Triton X-100 and bovine serum albumin

0006-8993/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2009.09.111

et al., 1994; Saldanha et al., 2000) and cowbird (Saldanha and Schlinger, 1997), and in mammals such as mice (Garcia-Segura et al., 1999; Ivanova and Beyer, 2000), rats (Sanghera et al., 1991; Garcia-Segura et al., 1999; Wehrenberg et al., 2001; Hojo et al., 2004; Rune and Frotscher, 2005; Munetsuna et al., 2009), monkeys (Yamada-Mouri et al., 1995; Wehrenberg et al., 2001; Yague et al., 2008) and humans (Sasano et al., 1998; Stoffel-Wagner et al., 1999). In these studies aromatase activity (Foidart et al., 1998; Garcia-Segura et al., 1999; Hojo et al., 2004) and the expression of the enzyme has been characterized by different techniques including RT-PCR (Yamada-Mouri et al., 1995; Sasano et al., 1998; Stoffel-Wagner et al., 1999; Ivanova and Beyer, 2000; Hojo et al., 2004; Munetsuna et al., 2009), *in situ* hybridization (Shen et al., 1994; Saldanha and Schlinger, 1997; Wehrenberg et al., 2001), Western blotting (Hojo et al., 2004) and immunohistochemistry (Sanghera et al., 1991; Garcia-Segura et al., 1999; Saldanha et al., 2000; Hojo et al., 2004; Rune and Frotscher, 2005; Prange-Kiel et al., 2006; Yague et al., 2008).

Several studies have employed RT-PCR to examine the expression of aromatase in the human brain, including the hippocampal formation (Sasano et al., 1998; Stoffel-Wagner et al., 1999). In addition, aromatase immunoreactivity has been characterized in the human hypothalamus and basal forebrain (Ishunina et al., 2005) and in the human temporal neocortex (Yague et al., 2006). However, to date the cellular distribution of aromatase in the human hippocampus remains unexplored.

In the present study we have assessed the distribution of aromatase immunostaining in normal hippocampal tissue taken from autopsies. In order to examine the expression of aromatase in particular types of neurons, we carried out dual immunohistochemical studies using antibodies against aromatase and against the calcium-binding proteins calbindin (CB), calretinin (CR) and parvalbumin (PV) that recognize specific populations of human cortical interneurons (DeFelipe, 1997). Furthermore, we investigated the expression of aromatase in glial cells, by combining aromatase immunohistochemistry with that for the glial fibrillary acidic protein (GFAP), as well as by combining aromatase labeling with that of a microglial marker, the LN3 antibody.

Finally, aromatase has been implicated in several aspects of epilepsy in experimental animals (Harden and MacLusky, 2004, 2005; Zhou et al., 2007). Moreover, the sclerotic hippocampus of epileptic patients presents impressive morphological and neurochemical changes involving both neurons (principal cells and interneurons) and glia (de Lanerolle et al., 1989; Sutula et al., 1989; Sloviter et al., 1991; Wittner et al., 2001; Arellano et al., 2004). Thus, we also analyzed the expression of aromatase in hippocampal surgical tissue obtained from patients suffering pharmaco-resistant temporal lobe epilepsy, to assess whether aromatase expression is modified in the epileptic hippocampus.

2. Results

2.1. *Normal hippocampus*

In the present study, we will consider that the hippocampal formation consists of the dentate gyrus (DG), the hippocampus proper (subdivided in three Ammon's horn fields: CA1, CA2 and CA3) and the subicular complex (see Fig. 1A for a panoramic view of sections immunostained for the neuronal marker NeuN). The pattern of aromatase immunostaining was similar in all the autopsy brain samples studied. Numerous aromatase-immunoreactive neurons were found in all hippocampal fields. The vast majority of aromatase-immunoreactive neurons were homogeneously labeled in both the perikaryon and proximal dendrites, such that many granule neurons in the DG (Figs. 2A and D) and pyramidal cells in the CA1–CA3 fields and subicular complex (Figs. 2B and C) were detected. In addition, occasional aromatase-immunoreactive axonal processes were identified (Fig. 6D). When the nuclei of aromatase-immunoreactive neurons were visualized, immunostaining was restricted to the perikaryon and excluded from the nucleus (Fig. 2D).

In addition to principal cells, aromatase was also observed in non-granule cells of the DG and in interneurons of the CA1–CA3 fields and subicular complex. This was verified by the co-expression of aromatase in different subpopulations of interneurons that were identified by their calcium-binding protein content (Fig. 3). In aromatase/PV double labeled sections, aromatase/PV-immunoreactive neurons were observed in all hippocampal fields (DG, Figs. 3A–C; CA1–CA3 fields, Figs. 3J–L). However, not all PV-positive neurons contained aromatase (Figs. 3J–L). Similarly, aromatase was expressed in some but not all CR-immunoreactive (Figs. 3D–F) and CB-immunoreactive (Figs. 3G–I) neurons in the DG and CA1–CA3 fields.

2.2. *Epileptic hippocampus*

Sclerotic hippocampal tissue from epileptic patients was characterized by neuronal loss, gliosis, granule cell dispersion and mossy fiber sprouting in the dentate gyrus, as well as by neuronal loss and gliosis in the stratum pyramidale of the CA1–CA3 fields (Arellano et al., 2004; Figs. 1B and 4). The pattern of aromatase immunostaining in the epileptic hippocampus varied in accordance with these cytoarchitectonic alterations, such that the amount of aromatase-immunoreactive neurons in all the hippocampal fields was reduced in parallel to the reduction in the number of surviving neurons. Furthermore, as in the autopsy tissue, aromatase immunoreactivity appeared to be located in the perikaryon and proximal dendrites of neurons in the epileptic hippocampus.

In the dentate gyrus, aromatase immunoreactivity was detected both in granule cells and in the dispersed cells of the molecular layer. Most labeled neurons displayed the typical DG granule cell morphology. In the CA1–CA3 fields and subicular complex, many aromatase-immunoreactive neurons had a typical pyramidal morphology (Fig. 5).

In accordance with the pattern of neuronal loss observed in NeuN immunostained sections (Fig. 1B), sclerotic areas showed a marked reduction of aromatase-immunoreactive neurons in the stratum pyramidale especially in the CA1 and CA3 fields. However, the pattern and intensity of aromatase immunostaining was apparently normal in non-sclerotic areas when compared with the autopsy material. Aromatase immunoreactivity was also detected in the surviving interneurons in the sclerotic hippocampus, including most of the surviving PV-immunoreactive neurons (Fig. 5).