Abstract—Estradiol biosynthesis is catalyzed by the enzyme aromatase, the product of the CYP19A1 gene. Aromatase is expressed in the brain, where it is involved not only in the control of neuroendocrine events and reproduction, but also in the regulation of neural development, synaptic plasticity and cell survival. In this review we summarize the existing data related with the detection of aromatase in human brain, with particular emphasis in the so-called “non-primary reproductive” areas. Besides hypothalamus, amygdala and preoptic/septal areas, aromatase is expressed in certain regions of basal forebrain, cerebral cortex, hippocampus, thalamus, cerebellum and brainstem of the human brain. Aromatase in human brain is produced by neurons, but there is also an astrocyte subpopulation that constitutively expresses the enzyme. The use of different methodological approaches, including the in vivo analysis by positron emission tomography of human subjects, has permitted to draw a general map of human brain aromatase, but the detailed distribution map is still far to be completed. On the other hand, despite the fact that there is only one aromatase protein, there are multiple mRNA transcripts that differ in the 5′-untranslated region, where regulatory elements reside. To date, some of the aromatase transcripts characteristic of cerebral cortex, as well as of human cell lines of neural origin, have been identified. This characteristic may confer tissue or even region-specific regulation of the expression and therefore it is conceivable to develop selective aromatase modulators to regulate the expression of the enzyme in the human brain.

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Key words: aromatase, estrogen receptors, estrogens, human brain, local estradiol synthesis.

Aromatase activity or expression has been described in several brain regions and different cell types in all vertebrates, from fish to primates (Calliard et al., 1977, 1978; Selmanoff et al., 1977; Steimer and Hutchison, 1980; MacLusky et al., 1986). Human brain aromatase activity was first described by Naftolin and collaborators in fetal hypothalamus (Naftolin et al., 1971a) and limbic system (Naftolin et al., 1971b). These findings perfectly matched with the role proposed for testosterone and estradiol in brain sex determination and the control of sex behavior (Harris and Levine, 1965; Westley and Salaman, 1976; McEwen et al., 1977; for reviews see Morris et al., 2004; Bakker and Baum, 2008; Wright et al., 2010). Thus, the concept of brain regions involved in the central reproductive control emerged. These regions include the hypothalamus, the preoptic area and the amygdala. Later on, alternative roles for sex steroids in the brain were discovered, acting in what it has been called “non-primary brain sex areas”. These new roles of sex hormones in the brain include the regulation of neuronal survival, the modulation of synaptic function and the control of neurogenesis (Azcoitia et al., 2001; Garcia-Segura et al., 2001; Kretz et al., 2004; Martinez-Cerdeno et al., 2006; Pawluski et al., 2009; see Garcia-Segura, 2008; Boon et al., 2010 for reviews). Moreover, it has also been described scenarios in which reproductive and non-reproductive areas converge to cope with unexpected roles. For instance, estradiol is part of the hormonal system modulating the amygdala-prefrontal cortex emotion circuit (Van Wingen, 2011); related to this, it is worthy to point out that both amygdala neurons (Roselli et al., 1998) and prefrontal cortex astrocytes (Luchetti et al., in press; Luchetti and Swaab, 2011) express aromatase. All these functions and others described in reproductive, skele-
In this review, we will focus mainly in the non-reproductive sphere of estrogens in brain. We will consider data on aromatase expression. For functional and gene regulation data, the reader is referred to recent reviews in the field (García-Segura, 2008; Roselli et al., 2009; Boon et al., 2010).

**AROMATASE EXPRESSION**

Brain aromatase has been evidenced by enzyme activity assays, protein expression (immunocytochemistry, Western blotting), mRNA expression (in situ hybridization and reverse transcriptase-polymerase chain reaction) gene transcription and binding of radioactive labeled inhibitors that generate positron emission tomography (PET) images. There is not a complete correlation in the results obtained with these different techniques, which could be explained in terms of methodological limitations, but also due to differences in stability of the products: for instance, short-lived transcripts that translate into stable protein or vice-versa; or protein modifications that can hide/expose the epitope against a particular anti-aromatase antibody.

**Enzyme activity assay**

One of the most extended assays is the in vitro radiometric method, based on the release of radioactive water after supplying radiolabeled (mostly tritiated) androgens to tissue homogenates (Weisz, 1982). This was the method used by Naftolin in the first descriptions of aromatase activity in human hypothalamus (Naftolin et al., 1971a) and limbic system (Naftolin et al., 1971b). Aromatase activity has been also demonstrated in hippocampus and cerebral cortex of non-human primates during late fetal and early postnatal life (MacLusky et al., 1987) and human temporal cortex from postmortem samples (Wozniak et al., 1998) and biopsies (Steckelbroeck et al., 1999). Cortical enzyme activity seems to be unrelated to sex or age differences (Steckelbroeck et al., 1999). The enzyme assay is also positive in human cell lines (Wozniak et al., 1998) and human astrocytomias (Weidenfeld and Schiller, 1984).

**Immunocytochemistry**

Aromatase immunoreactivity in human brain has been described in hypothalamus, preoptic area and amygdala (Ishunina et al., 2005). Besides these reproductive brain centers, aromatase has been localized in cholinergic basal forebrain (Ishunina et al., 2005) in temporal (Yague et al., 2006) and prefrontal cortices (Luchetti et al., in press), as well as in hippocampal formation, including both dentate gyrus and Ammon’s horn CA1–CA4 sectors (Ishunina et al., 2007; Yague et al., 2010). Aromatase immunoreactivity is reduced in the hippocampus of Alzheimer disease patients (Ishunina et al., 2007), but it remains intact in individuals affected by epilepsy (Yague et al., 2010). On the other hand, a clear increase in aromatase immunoreactivity has been detected in the basal forebrain (nucleus basalis of Meynert) during normal aging (Ishunina et al., 2005). Related to the effect of circulating estrogens in aromatase expression, there is not a complete study in humans, but it remains unaltered in non-human primates hippocampus, comparing intact versus ovariectomized females (Yague et al., 2008). This result is in agreement with the data of enzyme activity assay of human biopsies obtained during neurosurgical partial resection of the temporal lobe (Steckelbroeck et al., 1999, see former subsection).

With respect to cell types, mammalian brain aromatase is mostly expressed in neurons, but there are also some immunoreactive glia cells. Neurons expressing aromatase in the temporal cortex of human and non-human primates and in human hippocampus are both principal (pyramidal and dentate granule cells) and different types of interneurons (Yague et al., 2006, 2008, 2010). Neuronal aromatase is present in perikarya, dendrites and axons (Yague et al., 2006). Aromatase containing axon terminals establish synapses with both aromatase immuno-negative and immuno-positive dendrites and somata (Naftolin et al., 1996). Estradiol locally produced within the synapse is thought to regulate synaptogenesis (Kretz et al., 2004), neurotransmission (Balthazart and Ball, 2006) and synaptic plasticity (Mukai et al., 2010; Zhou et al., 2010).

Glia cells expressing aromatase in non-primate mammals include astrocytes in primary cultures (Azcoi-
tia et al., 2003), gliomas (Yague et al., 2004), cortical progenitor glia (Martinez-Cerdeno et al., 2006), or astrocytes in brain areas submitted to different types of lesion (Garcia-Segura et al., 1999, 2003). Human aromatase immunoreactivity in glia has been described in ependymal cells and choroid plexus ependymal (Ishunina et al., 2005), human glioblastoma cell lines (Yague et al., 2004) and prefrontal and temporal cerebral cortices (Yague et al., 2006; Luchetti et al., in press). Aromatase expression in human hippocampal glia has been described in astrocytes (Ishunina et al., 2007; Yague et al., 2010). Interestingly, hippocampal human astrocytes do not behave as those in other mammals and do not express the enzyme in certain stressful conditions, such as epilepsy (Yague et al., 2010).

mRNA expression

Besides some few studies in non-human mammals in which mRNA expression has been demonstrated by in situ hybridization (Roselli et al., 2001), the main body of data comes from reverse transcriptase-polymerase chain reaction (RT-PCR). First PCR descriptions of aromatase in human brain came from fetal material (Toda et al., 1994). In fact, the two key regulatory proteins of estrogen synthesis, namely the Steroidogenic Acute Regulatory protein (StAR), placed at the beginning of the pathway, and aromatase, at the end of the pathway, have been detected by PCR in human fetal brain (Pezzi et al., 2003). In adults, different aromatase transcripts have been found in several brain regions, including pons, thalamus, hypothalamus and hippocampus, of both women and men (Sasano et al., 1998). Stoffel-Wagner et al. (1998) found aromatase transcription in human temporal cortex. No differences between men and women have been described in temporal cortex (Yague et al., 2006; Luchetti et al., in press). Aromatase expression in human hippocampal glia has been described in astrocytes (Ishunina et al., 2007; Yague et al., 2010). Interestingly, hippocampal human astrocytes do not behave as those in other mammals and do not express the enzyme in certain stressful conditions, such as epilepsy (Yague et al., 2010).

Table 1. Summary of findings on aromatase and estrogen receptors distribution in human brain

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Aromatase</th>
<th>EAA</th>
<th>ICC</th>
<th>RT-PCR</th>
<th>PET</th>
<th>ERα</th>
<th>ERβ</th>
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<tbody>
<tr>
<td>Cerebral cortex</td>
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<td>3, 4</td>
<td>1, 3, 5</td>
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<td>7</td>
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<tr>
<td>Hippocampus</td>
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<td>8, 9</td>
<td>5, 10</td>
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<td>8, 11</td>
<td>7</td>
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<tr>
<td>Basal forebrain</td>
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<td>12</td>
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<tr>
<td>Basal ganglia</td>
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<tr>
<td>Preoptic area, hypothalamus and amygdala</td>
<td></td>
<td>14, 15</td>
<td>12</td>
<td>10</td>
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<td>7, 16</td>
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<tr>
<td>Thalamus</td>
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<tr>
<td>Cerebellum</td>
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<td>13, 18</td>
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<tr>
<td>Pons</td>
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<tr>
<td>Medulla</td>
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<tr>
<td>Gliomas</td>
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<td>19</td>
<td>20</td>
<td>4,20</td>
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<tr>
<td>Neuroblastomas</td>
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<td>4</td>
<td>18</td>
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</table>

of exons I.f, I.4 and PII and described also the exon I.3T (the truncated form of I.3) in human temporal cortex. They also analyzed aromatase transcription in the human cell lines glioblastoma multiforme T98G, glioblastoma-astrocytoma U373 and neuroblastoma SHSY5Y (Yague et al., 2004, 2006), confirming that all these cell lines transcribe aromatase. They use the same promoters identified in temporal cortex, that is, PII, I.3T, I.f and I.4 and a new one, the I.7 exon that could be a signature of transformed neural cells. The list of aromatase promoter exons seems not having an end, and recently it has been described the exon I.8, which makes the 11th in its class (Demura et al., 2008). Expression of this exon was extremely low in fetal human brain, but acquires certain significance in adults.

The mechanisms and factors that regulate the activity of the different promoters in the aromatase gene in the human brain need to be systematically explored. This will allow a selective regulation of aromatase ex-

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**Fig. 2.** Aromatase expression in cerebral cortex and hippocampus. Aromatase expressing cells appear through the entire human cortex (A), from layers 1–6 and in white matter (wm). Panel (B) depicts some pyramidal cells, in which aromatase is distributed in perikarya and processes. Rodent cortical pyramidal cells also express aromatase (C), but mainly restricted to layer 5. In human hippocampus, pyramidal cells (D), granular cells (F) and hilar interneurons (H), are aromatase immunoreactive. A similar pattern can be seen in rodent Ammon’s horn (E), but in dentate gyrus (G) only very few granular and hilar neurons are aromatase positive. CA1, sector 1 of Cornu Ammonis; gr, granular cell layer of dentate gyrus; h, dentate hilus; mol, dentate molecular layer. Scale bars: (A) $\sim 300 \, \mu m$; (B, C, E, H) $\sim 100 \, \mu m$; (G, F) $\sim 50 \, \mu m$; (D) $\sim 200 \, \mu m$. 

expression in different brain regions. In this aspect, there are already some data on aromatase transcription regulation in human glioma and neuroblastoma cell lines (Yague et al., 2009).

**Positron emission tomography (PET) imaging**

Brain aromatase can be evidenced *in vivo* by the use of radiolabeled inhibitors of the enzyme that specifically bind to it, allowing the analysis of aromatase distribution based on PET studies. Aromatase inhibitor selection is critical and certain third-generation inhibitors, such as letrozole that are currently used in clinics due to their high inhibitory activity, are not the most efficient, because of their relatively low affinity (Kil et al., 2009). Vorozole, a second generation inhibitor with a higher affinity (close to 15 fold), seems more appropriate for the analysis of brain aromatase distribution (Takahashi et al., 2006). Recently, Biegon et al. (2010) administered \([\text{N-methyl-}^{11}\text{C}]\) vorozole to six young, healthy voluntaries, three men and three women and observed the brain distribution of the radiotracer. They found that the higher content of aromatase was in thalamus, followed by amygdala, preoptic area and medulla oblongata. There was also a significant binding in temporal and occipital cortices, basal ganglia, cerebellum, pons and white matter.

**BRAIN ESTROGEN BIOSYNTHESIS IN THE HUMAN BRAIN: TO WHAT EXTENT?**

Comparing the heterogeneity in results obtained in different studies, the question is to determine how generalized is estrogen synthesis within the human brain. Is estrogen production only efficient when in large amounts, as in the hypothalamus, or even minute and extremely localized estrogen production matters, as it could be the case for synaptic plasticity modulation? Is the synthesis a sustained episode or rather a series of short events that could be misinterpreted as artifacts? Generally speaking, some molecular biology approaches, show a much restricted aromatase distribution than immunological methods, PCR, enzyme activity assays or PET. In a gene expression study in mouse with engineered aromatase co-expressing two reporter genes, positive signal was only found in preoptic

![Fig. 3. Aromatase expression in human cortical astrocytes. Colocalization of GFAP and aromatase can be seen in superficial (A) and deep (B) white matter associated to cerebral cortex. Most of the astrocytes in white matter close to cortical layer 6 (A) colocalize both proteins, while in deeper white matter (B), there are colocalizing cells (arrows), GFAP expressing only astrocytes (black arrowheads) and aromatase only expressing cells morphologically resembling astrocytes (white arrowheads). Similarly, only a fraction of interlaminar astrocytes of cortical layer 1 (C) colocalizes aromatase and GFAP (arrows); the most do not express the enzyme and few aromatase cells with astrocytic aspect are not GFAP stained (arrowhead). Scale bars: (A)≈50 μm, (B, C)≈100 μm.](image-url)
area, hypothalamus and amygdala (Wu et al., 2009). Similar results have been obtained by in situ hybridization studies (http://www.ncbi.nlm.nih.gov/gensat, Cyp19a1), but other molecular biology techniques, such as RT-PCR (Munetsuna et al., 2009), immunocytochemistry (Hojo et al., 2004), enzyme activity assays (Konkle and McCarthy, 2011), or PET images (Takahashi et al., 2006) confirm that aromatase is also present in many other brain areas. The generalized expression of aromatase can also be inferred by the fact that these mentioned areas accumulate estradiol, as measured by liquid chromatography followed by tandem mass spectrometry (Konkle and McCarthy, 2011). Furthermore, cerebral inhibition of aromatase, abolish brain-specific estrogen actions, such as hippocampal neuroprotection (Azcoitia et al., 2001), olfactory bulb neurogenesis (Veyrac and Bakker, in press), neuron survival (Hill et al., 2009), reduced reelin expression in Cajal-Retzius cells (Bender et al., 2010) or vestibular nuclei long-term potentiation (Grassi et al., 2009). To conclude, it is worthy to note that there is a positive correlation between aromatase expression and estrogen receptors distribution (Gillies and McArthur, 2010). This correlation is shown in Table 1 that summarizes the main findings on aromatase and estrogen receptors distribution in human brain.

The difficulties to obtain postmortem human material in appropriate conditions for the analysis is a limiting factor for a complete study of the areas implicated in estrogen production. Trying to reproduce in the human brain the results obtained in lower mammals can be a good start. In this regard, after finding aromatase immunoreactive neurons in cerebral cortex and hippocampus in rodents, we processed human cortex and hippocampus confirming that these areas were also aromatase positive in humans. Indeed, immunoreactivity is more generalized in human brain: as it is shown in Fig. 2, aromatase is mainly restricted to pyramidal neurons in layer V in rodent’s cortex, while in humans aromatase positive neurons are both pyramidal cells and interneurons, distributed through all cortical strata. Similarly, in rodent hippocampus the staining is particularly intense in apical dendrites of CA pyramidal cells but restricted to few granular cells, while in humans, CA cell bodies and most of the granular cells are immunopositive (Fig. 2). Special mention deserves glia: in non-primate brain, aromatase immunoreactive astrocytes are absent, unless a lesion had been induced, while in human brain, there are astrocytes that constitutively express the enzyme. For instance, some interlaminar astrocytes (Fig. 3), a subclass exclusive of primates that localize at cortical layer 1 (Oberheim et al., 2009), express aromatase, as do some astrocytes in deeper layers and in the white matter. These astrocytes are only a small fraction of the total astroglia population, and interestingly, some cells morphologically identical to astrocytes that do not express GFAP, are aromatase immunoreactive (Fig. 3).

Other glial cell types can be involved in estrogen synthesis;
Aromatase expression in rodent cerebellum and thalamus is also observed in human brain. In mouse cerebellum, only Purkinje cells are immunoreactive, while in humans the staining is observed in Purkinje cells and in interneurons (Fig. 4). With respect to the thalamus, several nuclei express aromatase in rodents, being reticular nucleus one of the deepest stained. Once again, human thalamus exhibits a corresponding immunoreactivity (Fig. 4) that perfectly matches with PET observations (Biegon et al., 2010). Finally, the high level of [N-methyl-11C] vorozole binding by inferior olivary complex observed in humans perfectly fits with the enhanced neurodegeneration in rat inferior olive after aromatase inhibition in an experimental lesion model (Sierra et al., 2003).

CONCLUSION
Local estradiol synthesis in the human brain seems to be a more extended phenomenon than initially supposed. However, a detailed map of the human brain regions expressing aromatase is still missing. It is also unknown whether different promoters of the aromatase gene are used in different brain regions to regulate aromatase expression. The use of non-invasive in vivo techniques of determination paralleled with the systematic study of aromatase expression in postmortem brain or brain biopsies is a mandatory first step to conduct thereafter a systematic RT-PCR analysis of the different transcripts of aromatase mRNA in different brain regions. At the same time, the mechanisms and factors that regulate the activity of the different promoters in the aromatase gene in the human brain need to be explored.

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