Journal of Steroid Biochemistry & Molecular Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Review

Control of aromatase in hippocampal neurons

Lars Fester[a], Nicola Brandt[a], Sabine Windhorst[b], Felicitas Pröls[a], Corinna Bläute[a], Gabriele M. Rune[a,*]

[a] University Medical Center Hamburg Eppendorf, Institute of Neuroanatomy, Martinistr. 52, 20246 Hamburg, Germany
[b] Department of Biochemistry and Signal Transduction, Martinistr. 52, 20246 Hamburg, Germany

ARTICLE INFO

Article history:
Received 3 July 2015
Received in revised form 1 October 2015
Accepted 8 October 2015
Available online xxx

Keywords:
17β-Estradiol
Aromatase
Neurons
Ca^{2+} transients
Aromatase phosphorylation
Aromatase immunohistochemistry

ABSTRACT

Our knowledge on estradiol-induced modulation of synaptic function in the hippocampus is widely based on results following the application of the steroid hormone to either cell cultures, or after the treatment of gonadectomized animals, thus ignoring local neuronal estrogen synthesis. We and others, however, have shown that hippocampus-derived estradiol also controls synaptic plasticity in the hippocampus. Estradiol synthesis in the hippocampus is regulated by several mechanisms, which are reviewed in this report. The regulation of the activity of aromatase, the final enzyme of estrogen biosynthesis, by Ca^{2+} transients, is of particular interest. Aromatase becomes inactivated as soon as it is phosphorylated by Ca^{2+}-dependent kinases upon calcium release from internal stores. Accordingly, thapsigargin dephosphorylates aromatase and stimulates estradiol synthesis by depletion of internal Ca^{2+} stores. Vice versa, letrozole, an aromatase inhibitor, phosphorylates aromatase and reduces estradiol synthesis. Treatment of the cultures with 17β-estradiol results in phosphorylation of the enzyme and increased aromatase protein expression, which suggests that estradiol synthesis in hippocampal neurons is regulated in an autocrine manner.

Contents

1. Introduction

Since the initial finding of Gould et al. [1], showing the loss of dendritic spines in the hippocampus after ovariectomy and its rescue after treatment of the animals with estradiol, a tremendous number of studies have demonstrated a role of estradiol in synaptic plasticity and cognition in the adult, as well as during development (for review see Spencer et al. [2]). *In vitro* studies, aimed at estradiol-induced modulation of synaptic function, commonly apply the steroid hormone to the cultures and to gonadectomized animals, but frequently neglect neuronal estrogen synthesis. Hippocampal neurons, however, express aromatase, the final enzyme of estradiol synthesis, and these neurons have been shown to synthesize and secrete 17β-estradiol [3,4]. The amount of estradiol in the hippocampal tissue varies with the estrus cycle and is higher in proestrus than in estrus animals, and even lower in hippocampal tissue of male animals [5,6]. Hence, the expression

* Corresponding author at: Institute of Neuroanatomy, University Medical Center Hamburg, Eppendorf Martinistr. 52, 20246 Hamburg, Germany.
Fax: +49 40 741054966.
E-mail address: rune@uke.de (G.M. Rune).

http://dx.doi.org/10.1016/j.jsbmb.2015.10.009
0960-0760/

and activity of aromatase is obviously controlled by, as yet, poorly defined mechanisms. For instance, in hippocampal slice cultures of female animals, application of estradiol induced spine synapse density only when hippocampal estradiol synthesis was experimentally reduced [7,8], pointing to a cross-talk of aromatase activity in the neurons and exogenously applied estradiol.

2. Functional roles of hippocampus-derived estradiol

In the hippocampus, locally synthesized estradiol maintains hippocampal synapses [9]. Inhibition of aromatase results in a decrease in spine density, spine synapse density, the expression of synaptic proteins in female mice and in female rat hippocampal slice cultures, and impaired long term potentiation [10–13] in acute and cultivated hippocampal slices. All effects could be rescued by application of estradiol [7]. Neurogenesis and axon growth are also inhibited after treatment of hippocampal cultures with letrozole [14,15]. In a recent report, we showed that systemic application of letrozole to ovariectomized female mice induced spine synapse loss in the hippocampus, underscoring the significance of locally synthesized estradiol on synaptic plasticity [16]. Studies on the role of aromatase with respect to hippocampus-related behavior are scarce (for review see: [17]). Estrogens have been demonstrated to enhance some aspects of cognitive function in animal and human models. However, the effects are often modest and inconsistent across studies (for review see: [18]). Findings from studies using the aromatase deficient mouse (ArKO) showed that ArKO mice of both sexes perform significantly worse than wild-type controls in tests for short-term memory [19]. In the Morris water maze test, ArKO females performed equally, if not better, than their wild-types counterparts, which may be due to the upregulation of NMDA receptors in these mutants [20]. This upregulation, however, did not occur after systemic treatment of wild-type mice with letrozole, the aromatase inhibitor [16]. Depressive-like behavior was demonstrated in ArKO mice [21], as well as an effect on the serotonin transporter, with behavioral consequences [22]. Memory deficits in women with breast cancer, and who are treated with aromatase inhibitors for therapeutical reasons, however, suggest a role of aromatase in learning and memory [23]. Bayer et al., [24] demonstrated that aromatase inhibition in women suffering from breast cancer induces hippocampus-related cognitive deficits, while no effects are observed in behavioral tasks which are related to the perirhinal cortex. Interestingly enough, letrozole treatment of mice had no effect on performance in the Morris Water maze, neither in female nor in male mice [13], while inhibition of hippocampal aromatization impairs spatial memory performance in male songbirds [25]. Rapid behavioral effects of estrogens and fast regulation of their local synthesis by brain aromatase, which shape complex behaviour, have been demonstrated, [26–29]. This is a strong indication that aromatase activity also influences hippocampus-related behavior in animals.

3. Activity and expression of aromatase in the hippocampus

The brain, like the adrenals, gonads and the placenta, is a steroidogenic organ. This paradigm emerged from studies carried out as early as the 1980s by Baulieu and co-workers; these studies showed that steroids such as pregnenolone and dehydroepiandrosterone were present in higher concentrations in the brain than in plasma (for review see: [30–34]). Furthermore, it was demonstrated that steroids persisted in the nervous system even after gonadectomy or adrenalectomy. Neurosteroids are defined as steroids that accumulate in the brain even in the absence of steroidogenic glands; they are synthesized in the brain from endogenous precursors by enzymes that are present *in situ* [35]. In this context, it is interesting that sex steroids, such as estradiol and testosterone, are not classically considered as brain-derived neurosteroids.

The substrate of all steroids, as well as neurosteroids, is cholesterol, and the equipment of the brain with enzymes of steroidogenesis makes a *de novo* synthesis possible [4,32,33,35]. In 1971, Roselli et al. [36] demonstrated the presence of aromatase, the final enzyme of estradiol synthesis, in the diencephalon for the first time. While others [37], using an in vitro radiometric assay, were unable to detect activity of aromatase in the rat hippocampus. MacLusky et al. [38] found activity of aromatase in explant cultures from mouse, rat, and in rhesus monkeys [39]. Beyer et al. [40] demonstrated immunoreactivity of aromatase in the rat hippocampus for the first time, followed by Beyer in chickens [41], and by Yague et al. in monkeys and humans [42,43]. On the mRNA level, Wehrenberg et al. [44] detected the expression of aromatase in rat hippocampus. Despite these early results, it still took some time before the synthesis and release was finally demonstrated [3,9,14,15].

Steroid Acute Regulatory Protein (StAR) is required to transport cholesterol into the mitochondria, where steroidogenesis starts by the conversion of cholesterol to pregnenolone. Using in situ hybridization and immunohistochemistry we demonstrated StAR mRNA expression, as well as StAR protein, in the hippocampus [45], thus confirming a previous study by Garcia-Segura et al. [46]. Although glial cells [47] and neurons express aromatase in the hippocampus (for review see: [48]), the coexpression of both proteins in neurons, which we found in our study, made *de novo* synthesis of estradiol in hippocampal neurons very likely. In fact, we were able to demonstrate a *de novo* synthesis of estradiol in dissociated cell cultures and in hippocampal slice cultures using the aromatase inhibitor letrozole, and by knockdown of StAR [14,15]. Studies by Kawato and coworkers [4,35] supported the hypothesis of a *de novo* synthesis of estradiol in the hippocampus. In addition, based on differences between StAR [45] and aromatase expression in hippocampal tissue sections, stronger nuclear expression of ERα in CA3 pyramidal neurons than in CA1 pyramidal neurons, and region-specific downregulation of synaptic proteins strongly demonstrate the autocrine/paracrine function of locally synthesized estradiol.

4. Regulation of hippocampal estradiol synthesis

Local concentrations of estrogens and aromatase may be classically regulated by gene transcription and enhanced protein synthesis [49–55]. We have previously shown that substrate availability and gonadotropins are potent regulators [8,56] of estradiol synthesis. Application of testosterone, the direct substrate of aromatase, and in particular cholesterol, tremendously upregulated estradiol synthesis in hippocampal cultures [8,57]. As a result, synapse density was increased in response to cholesterol and testosterone treatment of hippocampal slice cultures. The effect was abolished in the presence of letrozole, the aromatase inhibitor, showing the specificity of the estrogenic effect. Most importantly, gonadotropin releasing hormone (GnRH), of which the receptors are abundantly expressed in the hippocampus, upregulates estradiol synthesis dose-dependently in dissociated hippocampal cultures and in hippocampal slice cultures of female animals. As expected, treatment of hippocampal cultures with GnRH resulted in a consistent dose-dependent increase in the number of synapses and in the expression of synaptic proteins. Similar to our results on substrate availability, the increase in synapse density and in estradiol synthesis was abolished when aromatase was inhibited by simultaneous application of letrozole to the cultures [56].

Please cite this article in press as: L. Fester, et al., Control of aromatase in hippocampal neurons, J. Steroid Biochem. Mol. Biol. (2015), http://dx.doi.org/10.1016/j.jsbmb.2015.10.009