Acta Histochemica 117 (2015) 747–751

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

The relationship between estrogen receptors and microtubule dynamics in post-menopausal rat brain

Aksak Karamese Selina [a], Karamese Murat [b,*], Karakus Emre [c], Malkoc Ismail [d], Unal Bunyami [e], Gundogdu Cemal [f]

[a] Kafkas University, Medical Faculty, Department of Histology and Embryology, 36100 Kars, Turkey
[b] Kafkas University, Medical Faculty, Department of Microbiology, 36100 Kars, Turkey
[c] Ataturk University, Veterinary Faculty, Department of Pharmacology and Toxicology, 25240 Erzurum, Turkey
[d] Ataturk University, Medical Faculty, Department of Anatomy, 25240 Erzurum, Turkey
[e] Ataturk University, Medical Faculty, Department of Histology and Embryology, 25240 Erzurum, Turkey
[f] Ataturk University, Medical Faculty, Department of Pathology, 25240 Erzurum, Turkey

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 16 August 2015
Accepted 19 August 2015

Keywords:
Axonal neurodegeneration
Estrogen receptors
HPIP
Microtubule
Ovariectomy

ABSTRACT

Introduction: Estrogen is one of the most important regulators of neuron function. There is a broad consensus that a loss of estrogen is associated with neurodegeneration in the hippocampus which leads to cognitive impairment. Hematopoietic-Pbx-interaction-protein (HPIP) is a novel scaffolding protein which interacts with microtubules and estrogen receptors. In this study, we investigated the presence and role of HPIP in hippocampal neurons and examined the relationship between estrogen receptors and microtubule damage in post-menopausal rat brains.
Method: Eighty female Wistar albino rats, 12 weeks old, were divided into 10 groups: control, control + 17-β-estradiol, control + tamoxifen, control + mitogen-activated protein kinases (MAPK) inhibitor, control + phosphoinositide 3-kinase (PI3-K) inhibitor, ovariectomized, ovariectomized + 17-β-estradiol, ovariectomized + tamoxifen, ovariectomized + MAPK inhibitor, and ovariectomized + PI3-K inhibitor. Light and electron microscopic examinations were performed. Real-time polymerase chain reaction (PCR) was used to determine the expression level of HPIP in experimental groups.
Results: Light and electron microscopic examinations revealed morphological changes in hippocampal neuron axons. Axonal fluctuations and shrinkage were detected in all ovariectomized groups. HPIP was detected in all neurons with difference expression levels.
Conclusion: Proof that the HPIP protein can be found on hippocampal neurons may give rise to a new focus on neurodegeneration in post-menopausal women. Future molecular and pharmacological studies should be performed to reduce the rate of cognitive symptoms resulting from hippocampal neurodegeneration.

1. Introduction

Menopause is a normal stage in reproductive senescence in women which occurs around the age of 50 years and is characterized by the depletion of ovarian follicles, discontinuation of menstruation and a dramatic decrease in serum levels of estrogen, a female sex hormone which regulates the growth, differentiation and function of many reproductive tissues (Kato et al., 1998; Vandenakker and Glass, 2001; Zhang et al., 2013). It also affects neurons and other important cells and tissues, including heart, liver, and bone (Morissette et al., 2008).

Many cellular mechanisms, such as the development of neuron populations, neuronal excitability, axonal outgrowth, neuronal survival and neurogenesis are controlled by estrogen (McEwen, 2002; Wessler et al., 2006). Estrogen also influences cognitive and behavioral functions as well as the regulation of growth and the differentiation of axons (Jensen et al., 2010).

Estrogen operates through classical nuclear pathways and by signal-transduction cascades started at cell-surface membrane receptors. Estrogen diffuses into the cell then binds to the estrogen receptors ERα and ERβ, activating a downstream signaling cascade composed of protein–protein interactions and the DNA-binding actions of transcription factors (Gruber et al., 2004; Bjornstrom and Sjoberg, 2005). Some ERα and ERβ serine residues are critical for ligand-independent receptor activation induced by activating G-protein coupled receptors

* Corresponding author.
E-mail address: murat_karamese@hotmail.com (K. Murat).

http://dx.doi.org/10.1016/j.acthis.2015.08.005
0065-1281/

(GPERs); they become phosphorylated in response to secondary signaling pathways such as phosphatidylinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK), which play a key role in many classical signaling cascades (Lannigan, 2003). ERs are thought to activate G-protein coupled receptors to regulate L-type Ca^{2+} channels and activate protein kinase A (PKA), protein kinase C (PKC), and mitogen activated protein kinase (MAPK) signaling cascades (Coleman and Smith, 2001; Fu and Simoncini, 2008). ERα triggers these signaling pathways through interactions with well-characterized proteins like hematopoietic-Pbx-interacting-protein (HPIP) (Manavathi et al., 2006).

The cytoskeleton is an organized structure formed by the connection of actins, microtubules and intermediate filaments. These cytoskeletal structures play an important role in cell functions such as division, shape, structural integrity and cell motility (Ananthakrishnan and Ehrlicher, 2007).

Microtubule polymers are scaffold structures in the cells. They have the crucial role of transporting molecules from the neuronal cell body through the axons to the synapses. Dynamic stabilization of microtubules is necessary for the plasticity of the neuronal network. Any disturbance of this stability may have devastating consequences for brain function (Karamese et al., 2013).

Microtubules also have central roles in cellular functions such as protein transportation and cell proliferation, migration and division. They are required for regulating the signaling pathways between MAPK and extracellular signal-regulated kinases (ERK), and between PI3K and protein kinase B (PKB; also known as Akt). The function of microtubules in signal transduction has been clarified by recent studies which show that microtubules and various other classes of structural proteins may interact, demonstrating a relationship between microtubules and signaling cascades (Manavathi et al., 2006). The relationship between microtubule organization and structural genes, especially in neural-based learning and memory, has been shown by other studies (Unal et al., 2012).

HPIP, an 80-kD novel and scaffolding protein associated with microtubules and a leucine-rich domain, was first considered to be a repressor of the Hox-protein family and mediated-transcription processes. It was observed in some studies that HPIP has a potential to regulate ER signals by acting as a scaffold protein in the recruitment and processing of cell-survival signaling cascades PI3K and Src for cellular events in an intact microtubule network.

HPIP is often localized in the cytosol but it can also be found in the nucleus. It is thought to interact extensively with cytoskeleton fibers, especially microtubules (Manavathi et al., 2006; Karamese et al., 2013; Abramovich et al., 2000). It has been shown that HPIP may be a tether between ER and microtubules. Some studies showed that short-term estrogen treatment increased ER interaction with the HPIP–microtubule complex (Manavathi et al., 2006; Abramovich et al., 2000, 2002).

In this study, we investigated the presence and possible role of HPIP on hippocampal neurons as a model for neural degeneration and examined the relationship between estrogen receptors and microtubule damage in post-menopausal rat brains.

2. Materials and methods

2.1. Ethics statement

This project was approved by the Local Ethics Committee of Animal Experiments of Ataturk University, Veterinary Faculty with the number of B.30.2.ATA.0.01.02/1885.

Table 1
Detailed information about the experimental groups and used chemicals.

Groups	Doses	Administration	Duration
Cont (*n*:10)		Nothing was done	
Cont + Estr (*n*:10)	2 mg/kg 17-β-estradiol	Oral gauge	30 days
Cont + Tmx (*n*:10)	10 mg/kg tamoxifen	Oral gauge	30 days
Cont + MAPK (*n*:5)	1 μl/min/side U0126	Intrahippocampally using cannulae	1 min
Cont + PI3K (*n*:5)	1 μl/min/side Wortmannin	Intrahippocampally using cannulae	1 min
Ovx (*n*:10)		Nothing was done	
Ovx + Estr (*n*:10)	2 mg/kg 17-β-estradiol	Oral gauge	30 days
Ovx + Tmx (*n*:10)	10 mg/kg tamoxifen	Oral gauge	30 days
Ovx + MAPK (*n*:5)	1 μl/min/side U0126	Intrahippocampally using cannulae	1 min
Ovx + PI3K (*n*:5)	1 μl/min/side Wortmannin	Intrahippocampally using cannulae	1 min

2.2. Experimental groups

Eighty female Wistar albino rats, 12 weeks old and weighing from 180 to 200 g, were obtained from Atatürk University Medical Experimental Research and Application Center. Animals were kept in polycarbonate boxes at temperatures ranging between 19 °C and 22 °C, with a standard 12-h light–dark cycle.

The rats were randomly divided into 10 groups, called the control (Cont), control + 17-β-estradiol (Cont + Estr), control + tamoxifen (Cont + Tmx), control + MAPK inhibitor (Cont + MAPK), cControl + PI3-K inhibitor (Cont + PI3K), ovariectomized (Ovx), ovariectomized + 17-β-estradiol (Ovx + Estr), ovariectomized + tamoxifen (Ovx + Tmx), ovariectomized + MAPK inhibitor (Ovx + MAPK), ovariectomized + PI3-K inhibitor (Ovx + PI3K) groups. Forty female rats underwent bilateral ovariectomy to compose Ovx groups. The chemicals that were applied to the rats and the groups are detailed in Table 1.

Tamoxifen (Nolvadex-D, Astra Zeneca, UK) and 17-β-estradiol (Estrofem, Novo Nordisk, Denmark) were applied to specified groups for 30 days by oral gavage in appropriate doses. Rats were killed 24 h later from the last drug administration for tamoxifen and estrogen groups. U0126 (Promega, Madison, WI) and Wortmannin (Sigma–Aldrich, St. Louis, MO), MAPK and PI3-K inhibitors respectively, were injected intrahippocampally in the specified groups. Bilateral guide cannulae, aimed at the dorsal hippocampus (*x*: +3.8, *y*: −3.7, *z*: +3.6), were implanted. Stock solutions of Wortmannin and U0126 were prepared by dissolving in dimethyl sulfoxide (DMSO). All injections (1 μl/hippocampus) were performed within 1 min. The rats were then allowed to recover in their home cages for five to seven days. When all applications were finished, the rats were killed and the brains were removed prior to performing real-time polymerase chain reactions (PCR) as well as light and electron microscopic and electron microscopy.

The weight of all rats was recorded on the first day of the experiment and every 15 days following, at the same time (17:00–18:00), with the same precision scale (Precisa XB 3200C, Switzerland).

2.3. Light microscopic examination

After the brains were removed from the rats, all samples were rapidly fixed in 10% buffered formalin for 24–48 h. Samples were then dehydrated in a graded alcohol series, immersed in xylene and embedded in paraffin wax. Serial 5 μm thick coronal sections were cut using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany) following a systematic random sampling method. The sections were stained with hematoxylin–eosin.