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Expression of estrogen receptor β in hypothalamic stem cells

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Abstract

Neural stem cell activity at least partially accounts for the postweaning development of the sexually dimorphic nucleus of the preoptic area (SDN-POA) and estrogen selectively mobilizes neural stem cells in the 3rd ventricle stem cell niche (3VSCN). Here, we examined the expression of estrogen receptor β (ERβ) in the SDN-POA and the 3VSCN. A subset of cells within the SDN-POA--delineated with or without calbindin D28K (CB28)-immunoreactivity (ir)--exhibited ERβ-ir. The ependymal cells that expressed nestin within the 3VSCN also expressed ERβ. Interestingly, a few proliferating (Ki67 positive) cells within the 3VSCN and the hypothalamic parenchyma, including the SDN-POA, displayed ERβ-ir. In parallel, a subset of cells in the subventricular zone was double-labeled with nestin and ERβ or Ki67 and ERβ while the subgranular zone exhibited few such double-labeled cells. ERβ is expressed in hypothalamic stem cells that may regulate cell regenerative cycles.

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Introduction

Developmental estrogen treatment enlarges the sexually dimorphic nucleus of the preoptic area (SDN-POA) in male and female weanling rats [1]. Further, neural stem cell activity at least partially accounts for postweaning SDN-POA development [2, 3]. The hypothalamic subependymal niche is likely a heretofore undescribed source of neurogenesis [4] along with the rostral end of the 3rd ventricle, termed the 3rd ventricle stem cell niche (3VSCN). The 3VSCN appears distinguishable from other sections of the 3rd ventricle [2], such as the caudal portion, by its vigorous expression of nestin, a stem cell biomarker [5,6]. Notably, estrogen selectively mobilizes neural stem cells in the 3VSCN of postnatal day (PND) 21 rats, as evidenced by an increase in proliferative cell number and also an increase in mitotic activity [7]. Nevertheless, it remains unclear as to whether estrogen controls the differentiation of stem cells (stem cellàneuron-specific progenitor cellàCB28-expressing neurons) by which the size of the SDN-POA is determined [1,2].

Neural sex differences are under genetic regulation and regional sex differences are characterized by sexually dimorphic expression of neuronal proteins--such as calbindin-D28k (CB28/Calb1)--are theoretically driven by sex chromosome complement, steroid receptors or, in some instances, both [8]. CB28 has been used as a biomarker to define the SDN-POA [1] and stem cell activity is associated with the expression of the proliferative marker, Ki67. Ki67-immunoreactive cells exist in the SDN-POA of both weanling and adult rats [2]. The CB28 promoter is capable of conferring estrogen responsiveness [9] and nuclear expression of estrogen receptor beta (ERβ/Esr2). Recently, the nuclear expression of ERβ and Ki67 was compared between benign and malignant lesions and the results indicated that ERβ might inhibit proliferation [10]. Here, we examined ERβ expression in the 3VSCN and the SDN-POA to determine whether the ERβ-initiated pathway is involved in normal hypothalamic stem cell activity.

Materials and Methods

Animals. Weanling (PND 21, n=12) and adult (PND 110, n=8) male and female Sprague-Dawley rats were obtained from the National Center for Toxicological Research (NCTR) Breeding Colony. Animals were anesthetized using pentobarbital (i.p.) and sacrificed by intra-arterial perfusion with 100 ml of saline followed by 100 ml of 4% buffered paraformaldehyde. The brain was sectioned coronally into 30 µm thick slices and collected in series of three. All animal procedures were approved by the NCTR Institutional Animal Care and Use Committee.

Targeted brain regions/cells. Of initial interest was whether stem cells within the 3VSCN and the SDN-POA express ERβ. Two widely recognized neural stem cell reservoirs [i.e., the subventricular zone (SVZ) and the subgranular zone (SGZ)] served as within-subject controls: neural stem cells in these areas express the stem cell markers, Ki-67 and nestin. Similarly, granular cells in the hippocampal dentate gyrus served as within-subject positive controls for ERβ immunoreactivity [11].

Triple immunofluorescent labeling. A triple fluorescence labeling method described previously [7] was employed: label #1 (red fluorescent labeling), anti-ERβ antibody; label #2 (green fluorescent labeling), anti -calbindin D28K (CB28), anti-nestin, or anti-Ki67 antibody; and label #3 (blue fluorescent labeling) DAPI to delineate cellular nuclei. It is noteworthy that the DAPI-labeling demarcates the SDN-POA in a manner similar to that of CB28 [3]. The SDN-POA was defined by a DAPI-delineated nuclei-dense area and reference to three conventional landmarks: distance and characteristic orientation to the 3rd ventricle, the anterior commissure and the optic chiasm [3].

Results

As expected, most granular neurons in the dentate gyrus exhibited characteristic nucleic ERβ, defined either in 2D images or in stack-scanning video (3D, data not shown), allowing them to serve as within-subject positive controls for the ERβ-ir labeling. In addition, serving as the within-subject negative control, there was little ERβ-ir labeling in the 3rd ventricle cavity, where no tissue existed (cerebral spinal fluid within the cavity was lost during the tissue-preparation process, data not shown). As shown in Fig. 1 (B, D), a subset of cells within the SDN-POA territory expressed ERβ, regardless of whether they expressed CB28.

The granular neurons in the dentate gyrus displayed typical nucleic ERβ-ir (Fig. 2A & 2G). On the

other hand, the cells in the subgranular zone, whether they exhibited nestin-ir or Ki67-ir, were scarcely co-labeled with ERβ-ir (Fig. 2A & 2G). In contrast, a subset of neural stem cells or progenitor cells, double-labeled with nestin-ir or Ki67-ir, expressed ERβ in the SVZ (Fig. 2B & 2H). In the 3VSCN, a comparative number of ependymal cells expressing nestin were co-labeled with ERβ-ir (Fig. 2I). Interestingly, a subset of the Ki67-ir positive cells in the 3VSCN expressed ERβ (Fig. 2C2). In like fashion, a subset of the Ki67-ir positive cells within the SDN-POA territory expressed ERβ (Fig. 2F2).

Discussion

The novel findings in the present study include the demonstration of ERβ immunoreactivity in the 3VSCN, in both nestin- and Ki67-positive cells. Furthermore, a few of the Ki67-positive cells within the SDN-POA territory were also shown to express ERβ. Similarly, the positive control region for stem cell activity (i.e., the subventricular zone), displayed ERβ immunoreactivity in cells that expressed either nestin or Ki67.

ERβ is one of the major estrogen receptors and a member of the superfamily of nuclear receptor transcription factors, although its subcellular locations can be traced to not only the nucleus but also the cytoplasm and mitochondria. Conventional wisdom would indicate that in order for ERβ to participate in a given biological function, an estrogen ligand would first bind to the receptor and together both would then translocate into the cellular nucleus whereby the estrogen signaling pathway would be initiated [12,13]. ERβ, also classified as a steroid receptor (SR), is endowed with the characteristic features of a SR: it acts as a ligand-dependent transcription factor and its activity is associated with the cell cycle [14]. Thus, expression of ERβ at different stages within the cell cycle may regulate different biological functions. In the fully differentiated state (i.e., the G0 phase), activities of neurons expressing ERβ and residing in the SDN-POA or the dentate gyrus of the hippocampus would be relevant to the defeminization of sexual behavior [15] or to cognitive processes [16], respectively. On the other hand, activities of stem cells expressing ERβ (i.e., those in the 3VSCN) while in a quiescent cell cycle phase could be pertinent to inhibition or acceleration of those cells entering into the cell cycle.

Many stem cells within the SVZ, where cellular proliferative activity is vigorous, express ERβ while simultaneously displaying nestin-immunoreactivity (Fig. 2H), indicating that, by chance, many of those cells may be in a proliferative status. In contrast, nestin-immunoreactive cells within the 3VSCN also express ERβ, but it remains unknown whether they are stem cells and whether they are entering the cell proliferative cycle because there are is less stem cell activity in the 3VSCN (Fig. 2C) than in the SVZ (Fig. 2B) as estimated by the number of Ki67-positive cells. It is plausible that stem cells that express ERβ and that are in a proliferative status may function in either an inhibitory or an accelerative fashion to the process. In pathological states such as bladder cancer, activation of ERβ appears to promote growth of the cancer cells [17], whereas the role of ERβ in prostate lesions is thought to be anti-proliferative [18]. In the case of keratinocytes, activation of ERβ enhances cell proliferation in association with increased keratin-19 expression and decreased galectin-1 expression [19]. Collectively, it seems clear that tissue-specific effects of ERβ activation exist with regard to stem cell activities.

Presumably, progenitor cells expressing ERβ and in a proliferative status would participate in the promotion of differentiation. Neural stem cell proliferation and differentiation can be regulated by estrogen via receptor-dependent pathways [20]. Sex hormones, progesterone and 17β-estradiol, increase the differentiation of mouse embryonic stem cells to motor neurons in a receptor-dependent manner; however, ERβ appears not to be essential [21]. On the other hand, in an *in vitro* primate model terminally differentiated serotonergic neurons express ERβ but not ERα [22]. This suggests the existence of neuron-specific and/or timing-specific characteristics of neural stem cells or neural progenitor cells. Nevertheless, the manner in which the molecular signaling pathway(s) for estrogen function to control the process of converting neural stem cells into mature neurons that express specific biologically-functional proteins such as CB28 remains unknown

In conclusion, ERβ-ir seems a likely marker of estrogen-receptor associated pathways within the SDN-POA that subserve sexually-relevant behaviors and regulate cell regenerative cycles during proliferative periods (i.e., ERβ-Ki67 co-labeled cells). Because ERβ is heavily expressed in the 3VSCN in both nestin and Ki67-

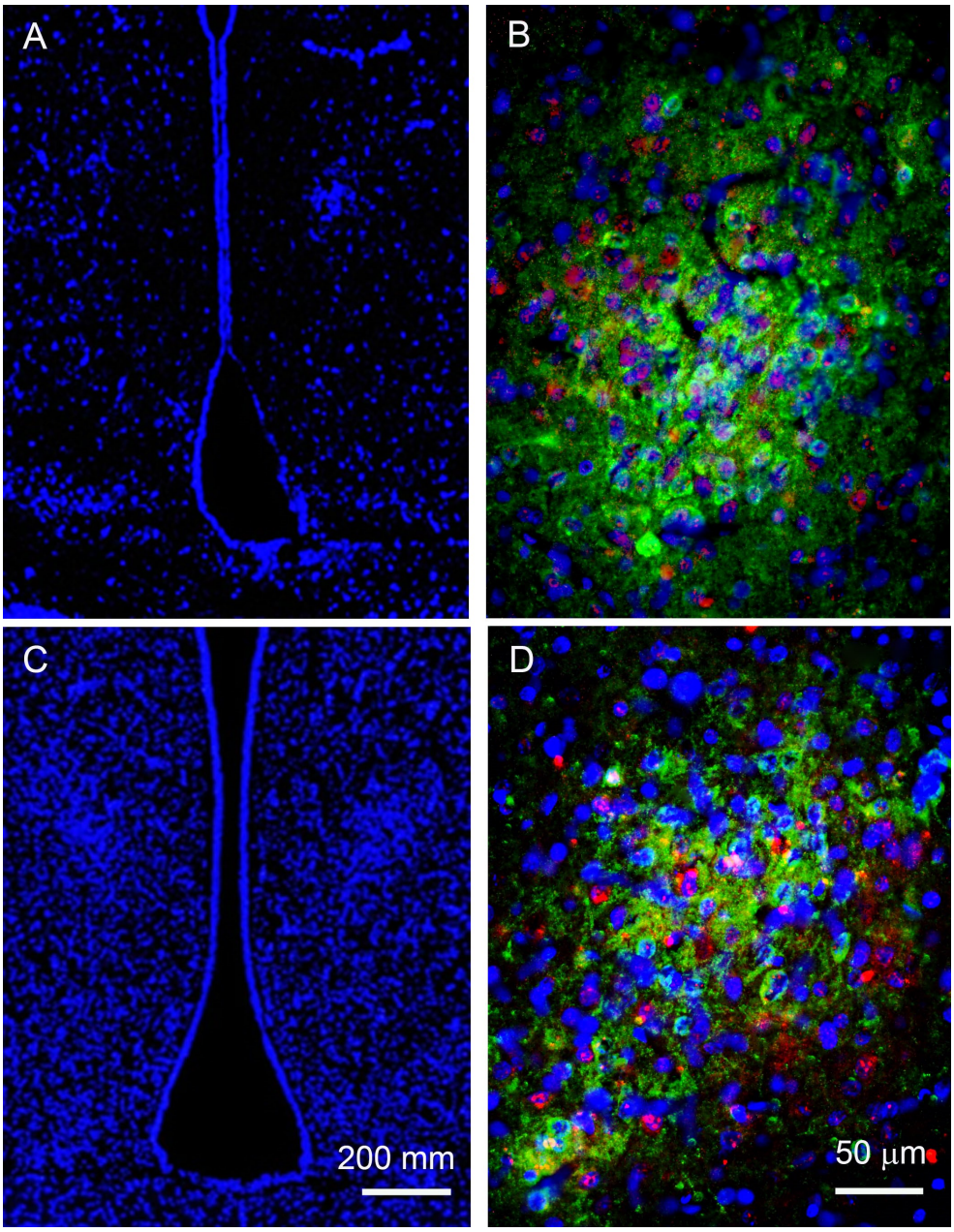

Fig.1. ERβ-immunoreactive (ir) cells in the sexually dimorphic nucleus of the preoptic area (SDN-POA). Images in the left column (A and C) show that the SDN-POA can be defined by its characteristic density (arrow-indicated) in association with the surrounding anatomical landmarks including the 3rd ventricle (3V) and the optic chiasm (OC). As shown in B and C, ERβ –ir localizes as spot-like deposits (red fluorescent label) mainly within the cellular nuclei, which are labeled with DAPI (blue fluorescent label). The CB28-ir cells are those that primarily display the intracellular green fluorescent signal. ERβ-ir cells in the SDN -POA can be either CB28-ir positive or CB28-ir negative. All images here were acquired from adult animals.

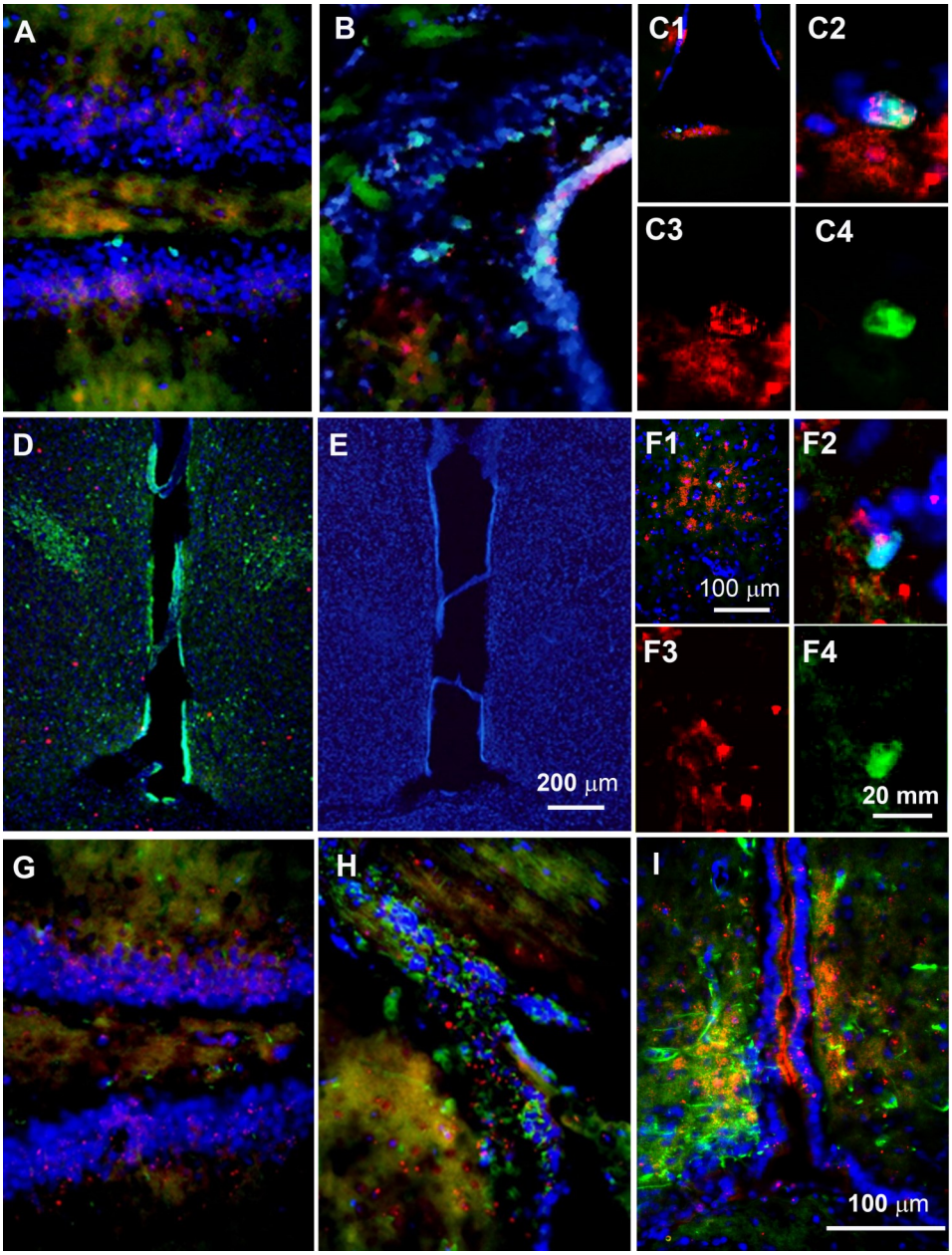

Fig. 2 ERβ-ir cells that express the stem cell markers Ki67 or nestin. Representative images are displayed for immunoreactivities for nestin or Ki67 (both with green fluorescence) and ERβ (red fluorescence) in the subgranular zone (SGZ: A&G) and the subventricular zone (SVZ: B&H). Both regions are well-accepted stem cell reservoirs in the brain. The granular neurons in the dentate gyrus (A&G), used here as a positive control, typically express ERβ (red). A subset of the Ki67-positive cells in the 3rd ventricle stem cell niche (3VSCN) express ERβ (C1), while the co-localization of Ki67-ir (C4) and ERβ (C3) is amplified in C2. Similarly, a subset of Ki67-ir cells express ERβ in the SDN-POA territory (F1) and the co-localization of Ki67-ir (F4) and ERβ (F3) is magnified in F2. Many ependymal cells in the 3VSCN express ERβ in association with nestin (I). Images A, B, G, H & I were acquired under the same magnification conditions and, thus, share the size bar in Image I. In similar fashion, C1 and F1 share the bar in F1; C2-4 and F2-4 share the bar in F4, and Image D and E share the bar in E. Noticeably in D, the green fluorescence indicates CB28 localization while the red fluorescence indicates Ki67. The images E and F1-4 were acquired from the same animal and adjacent to the tissue section presented in Image D (addressing Ki67-positive cells within territory of the SDN-POA); images F1-F4 were acquired from the tissue section presented in Panel E and represent magnified images. Using a similar strategy as presented in Fig.1A & C, Image E demonstrates that the Ki67-positive cells expressing ERβ are included within the SDN-POA territory identified by its characteristic density (arrow-indicated) in association with the surrounding anatomical landmarks including the 3rd ventricle (3V) and the optic chiasm (OC). All images in Fig. 2 were acquired from weanling rats.

positive cells, ERβ may play a role in the development of sexual dimorphism by regulating cellular proliferation in the sexually dimorphic structures surrounding the 3VSCN, including the SDN-POA.

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This document has been reviewed in accordance with United States Food and Drug Administration (FDA) policy and approved for publication. Approval does not signify that the contents necessarily reflect the position or opinions of the FDA. The conclusions in this review are those of the author(s) and do not necessarily represent the views of the FDA.

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