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Neuroscience Letters 392 (2006) 198–201
Neuroscience Letters
www.elsevier.com/locate/neulet

Estrogen contributes to structural recovery after a lesion

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Received 10 June 2005; received in revised form 1 September 2005; accepted 9 September 2005

Abstract

Over the last decade neuroscientists have accumulated a wealth of information confirming the trophic effects of 17β-estradiol (E2) on a variety of brain regions, such as the effects on hippocampal spine density, as well as other measures of structural reorganization. Here, we explore the hypothesis that E2 exerts a positive trophic effect on the cholinergic neurons of the basal forebrain, an area heavily implicated in memory and attentional processes. Female rats were ovariectomized at 3 months of age and lesioned with the immunotoxin 192 IgG-saporin before receiving a subcutaneous pellet containing .25 mg of estrogen or placebo, released over 60 days. The control, non-ovariectomized group was treated identically. At the end of the treatment, the brains were histologically prepared and we used image analysis procedures to evaluate changes in the dendritic arborization of surviving cholinergic neurons. As expected, infusion of the immunotoxin induced a reduction in dendritic arborization in all subjects, but was significantly different from control values only in ovariectomized rats. When differences within animals were factored in, dendritic size in ovariectomized animals treated with E2 was undistinguishable from intact controls. By contrast, in ovariectomized animals treated with placebo, dendritic length remained significantly reduced. These results suggest that E2 can not only protect but also reverse structural neurodegenerative processes in cholinergic neurons. Our data is particularly relevant in the context of female aging and postmenopausal dementia, since preserving an intact cholinergic system may be crucial to prevent at least some of the cognitive decline that occurs in Alzheimer's disease.

Keywords: Basal forebrain; Plasticity; Estrogen; Regeneration; Female rat

The effects of E2 on neuronal outgrowth and structural reorganization have been well documented. Among others, in vitro studies by Murphy and Segal [16] demonstrated that E2 has the ability to structurally reorganize hippocampal spine density, and Brinton et al. [3] have also shown that this steroid hormone can induce neurite outgrowth in cortical neurons. Previous work from our laboratory [6] has also demonstrated an enhancement in structural plasticity in cholinergic neurons from the basal forebrain, with a significant increase in total neuritic length and branch number in female cultures treated with E2 for 24 h, an effect not found in male cultures.

The ability of estrogen to enhance neuronal morphology has also been shown in vivo by Woolley and McEwen [21,22]. They reported that high levels of estrogen during the estrus cycle coincided with an increase in hippocampal spine density, and also that ovariectomy decreased spine density, an effect reversed by estrogen replacement. Whether estrogen's effect on hippocampal structural plasticity can potentially affect cognitive function is unknown, but work by Li et al. [10] suggests that E2-induced plastic changes in the hippocampus of mice coincide with an enhancement in spatial memory. Among others, work by Packard et al. [17] have also described a connection between E2 and memory by demonstrating that E2 treatment ameliorates cognition in rats when compared to untreated controls. The physiology of the basal forebrain cholinergic system is also modulated by E2. A number of studies have shown that E2 can significantly enhance the expression and activity of choline acetyltransferase, the synthetic enzyme responsible for the production of acetylcholine [15]. Consequently, the aim of our study is to determine whether E2 has the potential to structurally stabilize the morphology of cholinergic neurons after a partial lesion. This is particularly relevant in the context of female aging and postmenopausal dementia, since preserving an intact cholinergic system may be crucial to prevent Alzheimer's disease.

We obtained 16 female Fisher 344 rats, 3 months old, from the NIA colony at Harlan, of which 8 were ovariectomized

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doi:10.1016/j.neulet.2005.09.023

(OVX) and 8 left intact (NOVX). All experimental procedures were conducted with approved institutional animal care protocols and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23). Animals were housed individually in an environmentally controlled room with a 12 h:12 h light-to-dark ratio and ad libitum access to food and water. One week after arrival, all animals received a 200 nl injection of a solution of 192 IgG-saporin (0.075 mg/ml in PBS) into the HDB, using the following coordinates [18]: AP −0.6; DV −0.8 and RL +2.0. One of the OVX rats died after the surgery, leaving seven subjects in that group. Thirty days after surgery all animals were implanted with a subcutaneous pellet containing either 0.25 mg of E2 or placebo (P), generating four experimental groups: OVX + E2 ($n = 4$), OVX + P ($n = 3$), NOVX + E2 ($n = 4$) and NOVX + P ($n = 4$). These pellets are designed to release 4.167 μg of E2 daily for 60 days. The composition of the P pellets is identical except they lack the hormone. At the end of the treatment the rats were sacrificed by intracardial perfusion with 4% paraformaldehyde under deep anesthesia, the brains were extracted, and cut into 40 μm sections. The sections were immunocytochemically stained using a monoclonal antibody against the p75 receptor (clone 192; 1:7500 from Oncogene Science, MA, USA). After preincubation in a solution of PBS with 0.25% Triton X-100 and 3% H_2O_2 for 30 min to block endogenous peroxidase activity, sections were washed with 5% non-fat dry milk in PBS with Triton X-100 for 60 min at room temperature. Sections were then incubated overnight at 4 °C in the primary antibody, followed by several rinses with PBS and then incubation in biotinylated secondary antibody for 60 min. Sections were then washed in PBS and incubated in the ABC solution (Elite Kit, Vector Labs) at 1:100 dilution in PBS for an hour followed by further washes and a peroxidase reaction carried out with 0.05% 3-3′ diaminobenzidine (DAB, Sigma) and 0.01% H_2O_2. Sections were mounted on glass slides, dehydrated in a graded series of alcohol, cleared in xylene and coverslipped.

Analysis of arborization was performed using a Nikon inverted microscope with a motorized stage, coupled to a CoolSnapFX camera (Roper Scientific) and connected to a computer running MetaMorph software (v4.6r10 by Universal Imaging Corp.). Since animals were ipsilaterally lesioned, each served as its own control. Ten neurons were selected semi-randomly from each side of the brain (intact and lesioned) in all animals. Neurons were identified based on p75 immunoreactivity, an intact perikaryon and at least one dendrite, and were chosen based on their location along the perimeter of the HDB in a clockwise manner, beginning from the medial most aspect of the region. There was a bias in the selection, as we chose neurons situated in the periphery of the nucleus because they were easier to analyze since, comparatively, they were more free from visual obstruction caused by overlying processes from neighboring cells. To automate the photography, a standardized command (“macro”), was applied to each selected neuron. The best plane of focus was manually determined and the macro was then activated. The stage dropped to the bottom stop at −5.0 μm below that plane of focus and photographs were taken every 0.25 μm rising up through the best plane to 5.0 μm above that plane. The computer then processed the photographs to develop a composite image with all 41 photographs. The resulting image was converted to a standardized 8-bit TIFF (transfer image file format). A box 750 × 750 pixels was drawn around each selected neuron, and each dendritic segment was counted and its length calculated. The software included a calibration tool (0.348328 μm/pixel) and the measurements were taken directly in micrometers (μm). All measurements were verified by both a live image and 3D composite built from the source stack of 41 photographs. Statistics (un-paired *t*-tests and one-way ANOVA with post-hoc Fisher’s PLSD test) were performed

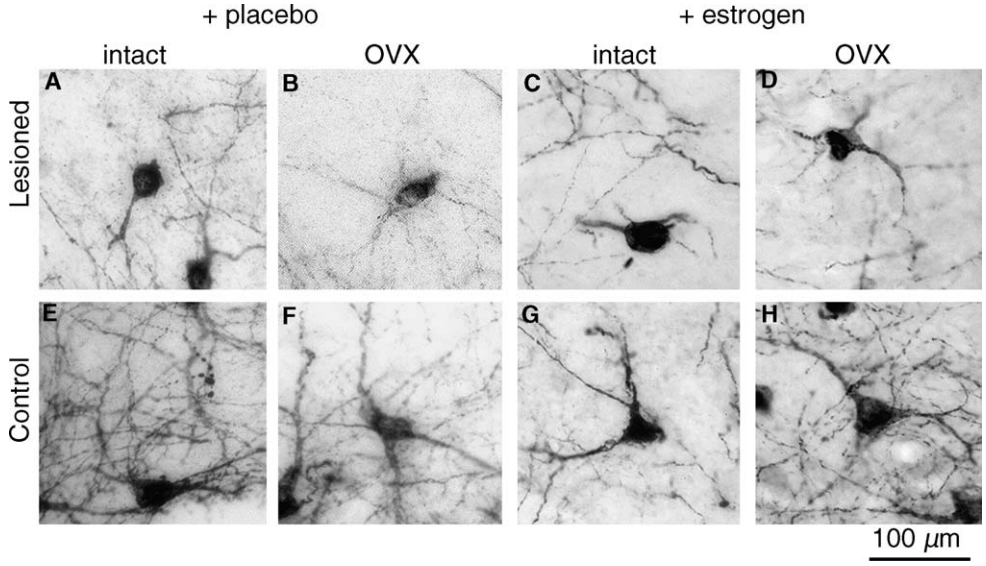

Fig. 1. Brightfield microphotographs of cholinergic neurons from the basal forebrain of four female rats (A–E; B–F; C–G; D–H). Sections were stained with an antibody against the $p75^{NTR}$ receptor, as described in the text. The top panels (A–D) illustrate neurons in the lesioned (192 IgG-saporin injected) side, and the bottom panels (E–H) correspond to the control (spared) side of the same animals. OVX: ovariectomized.