Neuroscience 228 (2013) 1–12

PI3K/AKT AND NF-κB ACTIVATION FOLLOWING INTRAVITREAL ADMINISTRATION OF 17β-ESTRADIOL: NEUROPROTECTION OF THE RAT RETINA FROM LIGHT-INDUCED APOPTOSIS

M.-S. MO,[a,b] H.-B. LI,[a] B.-Y. WANG,[a] S.-L. WANG,[a] Z.-L. ZHU[c] AND X.-R. YU[a,b]*

[a] *Department of Genetics and Molecular Biology, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China*

[b] *Environment and Genes Related to Diseases Key Laboratory of Education Ministry, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China*

[c] *Department of Ophthalmology, The Affiliated Guangren Hospital, Xi'an Jiaotong University, Xi'an 710004, China*

Abstract—The neuroprotective role of 17β-estradiol is well known; however, its mechanism of action remains unclear. In the present study, we applied light-induced apoptosis on the Sprague–Dawley rat retina to determine the neuroprotective effect of intravitreal administration of 17β-estradiol on retinal neurons and to demonstrate its underlying mechanism of action. Fourteen days after ovariectomy, adult female Sprague–Dawley rats received light damage. The functional and morphological changes of the retina were monitored by electroretinogram and hematoxylin and eosin staining, respectively. Retinal apoptosis was characterized by the presence of DNA laddering and positive terminal deoxyuridine triphosphate (dUTP) nick-end labeling. The phosphoinositide 3-kinase (PI3K)-specific inhibitor LY294002 was used to elucidate whether the PI3K/Akt signaling pathway was activated by 17β-estradiol. Western blotting was used to detect the activation of caspase 3 and Akt. Immunofluorescence was performed to determine the translocation of NF-κB. Our data showed that exposure to 8000 lux white light for 12 h resulted in functional damage to the rat retina, histological changes and retinal neuronal apoptosis. 17β-Estradiol significantly rescued retinal function by preventing neuronal apoptosis. Moreover, the inhibition of Akt activation by LY294002 increased retinal neuronal apoptosis, demonstrating that the PI3K/Akt signaling pathway is involved. Levels of cleaved caspase-3 were suppressed in the presence of 17β-estradiol, while LY294002 reversed the effects. It is noteworthy that NF-κB p65 also translocated from the cytoplasm to the nucleus after 17β-estradiol administration. This translocation was inhibited by pre-injection of LY294002. Taken together, these results indicate that 17β-estradiol intravitreal administration protects the function of the rat retina by preventing retinal neuronal apoptosis from light damage. In addition, the PI3K/Akt signaling pathway is activated, which inhibits caspase-3 activation and induces NF-κB p65 nuclear translocation.

Key words: 17β-estradiol, light damage, NF-κB, oxidative stress, PI3K/Akt, apoptosis.

INTRODUCTION

Abnormal apoptosis induced by environmental and intrinsic factors has been suggested to be involved in the onset of age-related neurodegenerative diseases (Dunaief et al., 2002). The severity and progression of age-related macular degeneration (AMD) increases following excessive exposure to environmental light (Taylor et al., 1990). Previous studies have shown that acute exposure to excessive light can induce retinal pigment epithelial cell and photoreceptor apoptosis (Marc et al., 2008), oxidative stress (Sparrow et al., 2002), autophagy (Kunchithapautham and Rohrer, 2007; Kunchithapautham et al., 2011) and biomacromolecule oxidation (Dentchev et al., 2007). Oxidative stress has been implicated as a main trigger for light damage demonstrated by the depletion of antioxidants and an increase of oxidative stress markers after light exposure (Strehlow et al., 2003). Moreover, some antioxidants such as thioredoxin and NG-nitro-L-arginine-methyl ester (Káldi et al., 2003; Tanito et al., 2005) have been reported to have significant effects on reducing the risk of retinal damage caused by excessive light.

17β-Estradiol (βE2) prevents neuron death induced by different insults such as oxidative stress (Stirone et al., 2005), glutamate excitotoxicity (Hilton et al., 2006), and serum deprivation (Gollapudi and Oblinger, 1999). In addition, βE2 protects neurons by increasing the number of phospho-CREB (cAMP-response element binding protein)-positive cholinergic cells (Szego et al., 2006), modulating the synthesis of cell survival proteins (Wen et al., 2004), and plays an anti-inflammatory role (Vegeto et al., 2008). In recent years, the protective effect of long-term treatment with βE2 has also been identified in Alzheimer's disease (Henderson, 2006; Carroll et al., 2007), Parkinson's disease (Xu et al.,

*Correspondence to: X.-R. Yu, Department of Genetics and Molecular Biology, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China. Tel/fax: +86-29-82655491.
E-mail address: xiaoruiy@mail.xjtu.edu.cn (X.-R. Yu).

Abbreviations: AMD, age-related macular degeneration; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; ERG, electroretinogram; FITC, fluorescein isothiocyanate; GCL, ganglion cell layer; HE, hematoxylin and eosin; IKK, IκB kinase; INL, inner nuclear layer; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segment; PBS, phosphate buffered saline; PI3K, phosphoinositide 3-kinase; SDS, sodium dodecyl sulfate; TUNEL, terminal dUTP nick-end labeling; βE2, 17β-estradiol.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.10.002

2006; Sarkaki et al., 2008) and ischemia–reperfusion injury (Wen et al., 2004). However, the detailed mechanisms still remain unclear. Additionally, the retina, as a direct extension of the central nervous system, arises from the same embryonic origin as the brain, in which βE2 also shows neuroprotective effects (Yu et al., 2004). An epidemiologic eye study of Los Angeles Latinos that included 5875 participants showed that exogenous estrogen could reduce retinal dysfunction and increase retinal pigment (Fraser-Bell et al., 2007). Other findings demonstrated that estrogen also has a protective role in neovascular AMD (Edwards et al., 2010), which includes protecting the retinal nerve fiber layer and increasing retinal blood flow (Deschenes et al., 2009). Furthermore, in the ganglion cell layer (GCL), estrogen exerts its neuroprotective role by affecting the synaptic connections between neurons (Kaja et al., 2003).

In the present study, intravitreal administration of βE2 was performed instead of intraperitoneal injection to obtain a higher local drug concentration in the vitreous cavity and reduce systematic side effects. Eight thousand lux-persistent light exposure for 12 h was applied to induce retinal neuron apoptosis, and to investigate the underlying neuroprotective mechanism of βE2 resistance to light-induced apoptosis. We confirmed that the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway plays a critical role in βE2 neuroprotection of retinal neurons in our model system, and further revealed that NF-κB and caspase-3 can be modulated by the PI3K/Akt signaling pathway.

EXPERIMENTAL PROCEDURES

Animals

All animals were cared for and handled according to the Xi'an Jiaotong University Guidelines for Animals in Research and the Association for Research in Vision and Ophthalmology statement for the use of animals in vision and ophthalmic research. Six- to seven-week-old female Sprague–Dawley rats (SPF Laboratorial Animal Center, Xi'an Jiaotong University, Xi'an, China), each weighing 180–230 g, were housed in a temperature-controlled room. Animals were maintained on a 12-h light/12-h dark schedule (lights on at 7:00 am) with a cage lighting level of 60 lux and had free access to food and water. Rats were ovariectomized 2 weeks before use.

Light damage

Ovariectomy as described previously (Green et al., 1999) was performed on female rats 14 days before light damage. Rats in cages (one rat per cage) were exposed to diffuse, cool, white fluorescent light coming from the top of the box, which contained eight 40-W white fluorescent tubes (Philips, Eindhoven, The Netherlands) and light sources were suspended 30 cm above. The light damage apparatus contained four positions, in different positions the deviation of light intensity was less than 5%, and the rats were randomly placed at the above positions. A light meter (TES-1332A, TES Electrical Electronic Corp., Taipei, Taiwan) was used to measure the average luminance (4000 or 8000 lux) on the cage floor. Ventilation facilities were used to maintain the temperature of 22–24 °C and relative humidity of 50–60%. The water bottle was placed in an appropriate position on the cage cover and food was placed in the cage to avoid blocking light exposure. Before light damage, rats were dark-adapted for 12 h to elevate retinal light damage sensibility. Different light intensities and exposure durations were compared to select the best experimental conditions. After light exposure, rats underwent 24 h dark recovery and electroretinogram (ERG) tests were performed. Rats were sacrificed using CO_2, enucleation of the eyes was carried out immediately, and the retina was dissected and frozen at −20°C or fixed with 4% (w/v) paraformaldehyde for structural studies. Some rats were continually kept in normal light conditions for 7 days to analyze the evolution of retinal damage.

Intravitreal administration

Ovariectomized female rats were deeply anesthetized with an intraperitoneal injection of ketamine (120 mg/kg body weight) and xylazine (6 mg/kg body weight) after 18-h dark adaptation. Rats were intravitreally injected with 4 μl of 10^{-6} M, 10^{-5} M and 10^{-4} M βE2 (Sigma Chemical Co., St Louis, MO, USA) to compare the extent of neuroprotection. Intravitreal administration was performed in a darkened room under red light illumination to preserve dark adaptation. Because 4 μl of 10^{-5} M βE2 was found to be the most effective dose for preventing neuronal apoptosis, all subsequent experiments used 10^{-5} M βE2. Rats were divided into four groups. Before light injury, rats were intravitreally injected with 4 μl saline, 4 μl of 10^{-5} M βE2 (dissolved in saline), and 4 μl of 10^{-5} M LY294002 (LY, Cell Signalling Technology Inc., Danvers, MA, USA) (dissolved in saline), and an additional group was intravitreally injected with 2 μl of 2×10^{-5} M LY (dissolved in saline) 30 min before 2 μl of 2×10^{-5} M βE2 (dissolved in saline) in the same eye. After administration of treatments, rats had 4 h to recover before light damage.

DNA ladder assay

The DNA ladder assay was performed after the 24-h dark recovery period. The genomic DNA purification kit (Boster Biological Technology Co., Wuhan, China) was used to extract DNA according to the manufacturer's instructions. Electrophoresis was carried out on a 2% (w/v) agarose gel. Images were captured under a UV light with the SynGene gel documentation system (SynGene-Synoptics, Cambridge, UK).

Electroretinogram tests

ERGs were recorded with an ERG recording system (TGC-350v, Chongqing Tektronix Corp., Chongqing, China) to measure the function of surviving photoreceptors. After dark adaptation, ERG values were taken before and after 24-h constant light exposure. The rats were deeply anesthetized with an intramuscular injection of ketamine (120 mg/kg body weight) and xylazine (6 mg/kg body weight). At 30 min before anesthesia, one drop of 1% (v/v) tropicamide (Mydrin-P®; Santen, Osaka, Japan) was applied to the cornea to dilate the pupil, and one drop of 0.5% (w/v) proparacaine HCl (Santen Pharmaceutical Co., Osaka, Japan) was applied as a local anesthetic. Full-field ERGs were recorded from both eyes using gold wire electrodes placed centrally on the cornea. The different electrodes were connected to a two-channel amplifier. A monopolar reference electrode was placed on the buccal mucosa and the ground electrode on the tail of the rats. According to the International Clinic Visual Electrophysiological Standard Program, we used 1.66 $cd \cdot ts/m^2$ as the luminous energy in Rod-ERG and 3.32 $\log cd \cdot ts/m^2$ in Max-ERG through a Ganzfeld, the stimulus frequency was 0.2 Hz for the Rod-ERG and 0.5 Hz for the Max-ERG, and the responses