Doi: 10.1111/j.1742-7843.2007.00044.x
Journal compilation © 2007 Nordic Pharmacological Society. *Basic & Clinical Pharmacology & Toxicology*, **100**, 214–216

Short Communication

Neuroactive Steroids Protect Retinal Tissue through σ_1 Receptors

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(Received October 20, 2006; Accepted December 7, 2006)

The term 'neuroactive steroids' has been adopted for steroids, including 17β-oestradiol and dehydroepiandrosterone-sulphate (DHEA-S), that might alter neuronal excitability through the cell surface through interaction with specific neurotransmitter receptors. It has been shown [1] that administration of 17β-oestradiol exerts protective effects against ischaemic damage in rat retina. Recently, we demonstrated [2] that 17β-oestradiol and DHEA-S inhibit biochemical changes induced by ischaemia injury in rat retina and that these effects were antagonized by σ_1 receptor blocker pre-treatment. More recently, we showed [3] that neuroactive steroids protect retinal pigment epithelium against oxidative stress and that this effect was blocked by pre-treatment with σ_1 receptor antagonist. The mechanism by which neuroactive steroids exert these protective effects remains unclear. A direct interaction between neuroactive steroids and σ_1 receptors has been hypothesized from the finding that several steroids inhibit the binding of σ_1 receptor radioligands *in vitro* and *in vivo* [4]. σ_1 Receptors are a unique class of non-opioid, non-phencyclidine-binding sites heterogeneously distributed in the nervous system and in peripheral organs that may serve as receptors for any, as yet unidentified, endogenous ligand. Recently, the presence of σ_1 receptors in rat Müller cells, rat ganglion cells and human retinal pigment epithelial cells have been demonstrated [5], in spite of the functional role of σ_1 receptors not yet being clearly determined. The present study was designed to determine whether 17β-oestradiol and DHEA-S protect rat retinal tissue against ischaemia/reperfusion damage and whether σ_1 receptors are involved in the mechanism of action.

Materials and methods. Male Sprague-Dawley rats weighing 250–300 g were used. Animal procedures were approved by the Animal Care and Use Committee of the University of Catania (Catania, Italy). Preliminary studies in our laboratory under the same experimental conditions did not show any gender differences. Retinal ischaemia/reperfusion model has been performed as described previously [2]. Briefly, rats were anaesthetized and the anterior chamber was cannulated with a 30-gauge needle attached to a raised saline reservoir. Retinal ischaemia was induced by elevating the intraocular pressure to 120 mmHg for 45 min. A hand-held ophthalmoscope was used to visually inspect the retinal blood vessels and verify ischaemia. After 45 min., the saline reservoir was lowered and the intraocular pressure and retinal circulation was allowed to return to normal over a period of 10 min. The cannula was removed from the cornea and the animals were allowed to recover. Rats were injected (0.1 and 1 mg/kg intraperitoneally) with 17β-oestradiol, DHEA-S, PRE-084 (σ_1 receptor agonist), 30 min. prior to the transient ischaemic insult, with or without a pre-treatment with BD1047 {[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(diamino)ethylamine} (σ_1 receptor antagonist) 45 min. prior to the ischaemia damage. The dose of compounds used in this study has been chosen on the basis of previous work [2]. After ischaemia, the rats were left to recover for 7 days (reperfusion), then the animals were killed, and the globes were fixed in 1% gluteraldehyde and 4% formalin in 0.1 M phosphate buffer (pH 7.4; overnight at 4°C), and embedded in paraplast. Five-micrometer thick sections were cut through the optic disc of the eye and stained with haematoxylin/eosin. The retinal layers in each section at a distance of approximately 1.5 mm of either side of the centre of the optic nerve head were recorded onto hard-disk as image data using a digital camera connected to a light microscope. The thickness of two different retinal layers was measured to evaluate ischaemic retinal damage: (i) the inner retinal layer, which extends from the inner limiting membrane to the boundary of the outer plexiform layer and the outer nuclear layer and (ii) the inner plexiform layer. Data were analysed using Newman-Keuls test. Statistical significance was accepted at a level of $P < 0.05$.

Results. As indicated by the morphometric analysis, the inner retinal layer and the inner plexiform layer in the ischaemic eye of vehicle-treated group were thinner than in the normal eye at 7 days after reperfusion (table 1). Representative photomicrographs demonstrating ischaemia-induced retinal

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Table 1.

Effects of 17β-oestradiol, DHEA-S and PRE-084 on ischaemia-induced retinal damage. Measurements of thickness of retinal layers 7 days after ischaemia.

Treatment	Dose (mg/kg i.p.)	Thickness (μm)	
		IRL	IPL
CTR–	–	103.1 ± 3.2	49.3 ± 1.9
CTR+	–	71.2 ± 2.0	24.2 ± 1.3
17β-oestradiol	0.1	84.0 ± 1.2*	43.3 ± 1.8*
17β-oestradiol	1.0	92.1 ± 2.0†	50.3 ± 2.0†
DHEA-S	0.1	81.4 ± 1.9*	39.9 ± 1.5*
DHEA-S	1.0	89.8 ± 2.5†	48.3 ± 1.3†
PRE-084	0.1	80.5 ± 2.7*	40.0 ± 2.0*
PRE-084	1.0	88.5 ± 3.0†	46.7 ± 1.3†
17β-oestradiol + BD1047	0.1	72.5 ± 1.0	25.6 ± 2.8
17β-oestradiol + BD1047	1.0	70.9 ± 1.5	28.5 ± 2.4
DHEA-S + BD1047	0.1	73.0 ± 2.5	26.6 ± 1.4
DHEA-S + BD1047	1.0	71.8 ± 3.0	29.0 ± 3.3
PRE-084 + BD1047	0.1	74.0 ± 3.2	23.8 ± 3.8
PRE-084 + BD1047	1.0	73.3 ± 2.6	26.0 ± 2.9

CTR–, normal retina; CTR+, ischaemic retina of vehicle-treated group; IRL, inner retinal layer; IPL, inner plexiform layer. Data are expressed as mean ± S.D. for 6–10 rats. *$P < 0.05$; †$P < 0.001$ versus CTR+.

change 7 days after reperfusion are shown in fig. 1. Treatment with 17β-oestradiol and DHEA-S significantly reduced the retinal damage in a dose-related manner. Although the σ_1 receptor agonist PRE-084 significantly prevented retinal damage, the magnitude of its effect was lower compared to the neuroactive steroids. The effects of neuroactive steroids and PRE-084 were antagonized by the pre-treatment with the σ_1 receptor antagonist (BD1047). The effect of BD1047 alone was tested, and we did not show any modification in terms of retinal thickness (data not shown). Sham-operated rats (subjected to the same surgery except the intraocular pressure increase) did not show any change in terms of retinal thickness (data not shown).

Discussion. The present results show that 17β-oestradiol and DHEA-S reduced the damage induced by ischaemia reperfusion in rat retina and suggest that the retinal tissue protection could be mediated by activation of σ_1 receptors. These results are in accord with our previous observations [2] on retinal ischaemia showing that neuroactive steroids were able to inhibit lactate accumulation and induced an increase in glucose and adenosine triphosphate content in retinal tissue after ischaemic insult. The results of this previous study [2] demonstrated that the protective effects of 17β-oestradiol and DHEA-S on ischaemia reperfusion injury in the rat were mediated, at least in part, by activation of σ_1 recognition sites. In fact, 17β-oestradiol and DHEA-S were able to inhibit lactate accumulation and induce an increase in glucose and adenosine triphosphate content in retinal tissue, and these effects were partially or completely antagonized by a σ_1 receptor antagonist [2]. Direct investigation of the protective effects of oestrogen against ischaemia reperfusion-induced retinal damage demonstrated that 17β-oestradiol reduces leucocyte accumulation and consequent retinal damage, particularly in the inner retina [6]. More recently, administration of 17β-oestradiol has been shown to protect the inner retina from apoptosis and early changes in synaptic connection associated with ischaemia [1]. We have shown that the two neuroactive steroids have different efficacy in comparison to the σ_1 receptor agonist, particularly the 17β-oestradiol. We hypothesize that 17β-oestradiol acts, at the same time, through other mechanisms. It has been demonstrated [6] that intraperitoneal pre-treatment with 17β-oestradiol (0.1 mg/kg) attenuates retinal ischaemia reperfusion injury in rats on 48 hr after reperfusion, likely through the inhibition of leucocytes accumulation. Furthermore, it has been shown [7] that the protective effects of 17β-oestradiol against oxidative stress using retinal neurones is not mediated through activation of oestrogen receptors. It is also worthwhile to highlight that 17β-oestradiol may interfere with glutamate receptors protecting the neurones from *N*-methyl-D-aspartate (NMDA) insult [8]. Based on these considerations we can not rule out that other mechanisms are involved in the retinal protective process of neuroactive steroids. In general neuroactive steroids in man decline with age, and likely this is linked to the onset of age-related diseases (e.g. age-related macular degeneration). These novel findings clearly point to an important role of neuoractive steroids for the neuroprotection of retinal tissue, suggesting that they could represent the endogenous effectors for the σ_1 receptor, and indicating that they may offer promise for the treatment of retinal degenerative diseases.

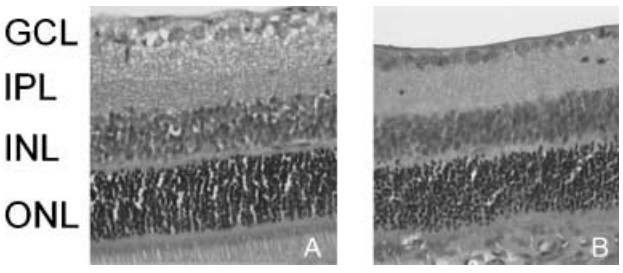

Fig. 1. Representative photomicrographs showing ischaemia-induced retinal change 7 days after reperfusion. (A) Normal eye; (B) ischaemic eye. Original magnification ×40. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer.

Acknowledgements

This work was supported by a grant from Ministero Istruzione Universitá e Ricerca, Misura III.4 Programma Operativo Nazionale Ricerca Scientifica e Tecnologica.

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