Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 498 (2004) 111–114

www.elsevier.com/locate/ejphar

Short communication

Effects of neurosteroids on ischemia–reperfusion injury in the rat retina: role of σ_1 recognition sites

Claudio Bucolo, Filippo Drago*

Department of Experimental and Clinical Pharmacology, School of Medicine, University of Catania, Viale A. Doria 6, Catania I-95125, Italy

Received 17 May 2004; accepted 17 June 2004
Available online 20 August 2004

Abstract

The effects of neurosteroids, 17β-estradiol and dehydroepiandrosterone-sulfate (DHEA-S), were investigated on retinal degeneration using a rat model of ischemia–reperfusion injury. The animals were anaesthetized and retinal ischemia was induced by elevating the intraocular pressure to 120 mm Hg for 45 min. Neurosteroids were injected intraperitoneally before ischemia and immediately after reperfusion. Retinal biochemical changes such as increase of lactate content and decrease of glucose and ATP were significantly inhibited by neurosteroids compared to the control ischemic group. The effects of 17β-estradiol and DHEA-S were antagonized by pre-treatment with the σ_1 site antagonist. These findings suggest that 17β-estradiol and dehydroepiandrosterone-sulfate may affect the metabolic state of surviving neurons and glial cells after ischemic injury and that they act, at least in part, through involvement of σ_1 recognition sites.

Keywords: Neurosteroid; σ_1 Recognition site; Retina; Neuroprotection

1. Introduction

Neurosteroids, including 17β-estradiol and dehydroepiandrosterone-sulfate (DHEA-S), represent steroid hormones that are synthesized de novo in the brain and act locally on the cells of the central nervous system (CNS). The term "neurosteroids" has been adopted for steroids that might alter neuronal excitability via the cell surface through interaction with specific neurotransmitter receptors. While the action of steroids at the level of the genome requires a period of time that lasts from minutes to hours and is limited by the rate of protein biosynthesis, the modulatory effects of neurosteroids occur during a millisecond to second period of time. Thus, the genomic and nongenomic effects of steroids within the CNS provide the molecular basis for a broad spectrum of steroid actions on neuronal function and plasticity. Recent studies have indicated that the administration of 17β-estradiol exerts protective effects against ischemic damage in rat retina (Nonaka et al., 2000; Kaja et al., 2003). Furthermore, it has been demonstrated that DHEA-S protects hippocampal neurons against excitatory amino acid-induced neurotoxicity, and prevents oxygen-glucose deprivation-induced injury in cerebellar granule cell cultures (Kimonides et al., 1998; Kaasik et al., 2001). The mechanism or the mechanisms by which neurosteroids exert these protective effects remain unclear. A direct interaction between neurosteroids and σ_1 receptors has been hypothesized from the evidence that several steroids inhibit the binding of σ_1-receptor radioligands in vitro and in vivo (Maurice et al., 2001). The term "sigma" (σ) is used to refer to a unique class of nonopioid, nonphencyclidine-binding sites heterogeneously distributed in the CNS and in peripheral organs that may serve as receptors for any, as yet unidentified, endogenous ligand (Walker et al., 1990; Quiron et al., 1992; Leitner et al., 1994). According to biochemical and radioligand-binding data, σ recognition sites have been classified into at least two types, termed σ_1 and σ_2 (Quiron et al., 1992). A σ_1 binding protein was cloned (Hanner et al., 1996), and its sequence shows significant similarities to sterol C_8–C_7 isomerases from fungi. The functional role of σ recognition sites has not yet

* Corresponding author. Tel.: +39 95 7384236; fax: +39 95 7384238.
E-mail address: fdrago@tin.it (F. Drago).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.06.067

been clearly determined. The present study was designated to investigate the effects of 17β-estradiol and DHEA-S against ischemia–reperfusion injury in rat retina, and to elucidate the role of σ_1 recognition sites in the effects of neurosteroids.

2. Material and Methods

Male Sprague–Dawley rats weighing 250–300 g (Charles River, Calco, Italy) were used. Animal procedures followed the guidelines of the Animal Care and Use Committee of the University of Catania, and conformed to the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in research. The compounds 2-(4-morpholinethinyl)-1-phenyl-cyclonexane carboxylate hydrochloride (PRE-084) and *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino) ethylamine (BD1047) were purchased from Tocris (Avonmouth, UK) and all other compounds from Sigma-Aldrich (St. Louis, MO, USA). Rats were anaesthetized with ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). Pupils were dilated with a topical application of 1% tropicamide (Visumidriatic, Visufarma, Milan, Italy). The anterior chamber of the left eye was cannulated with a 30-gauge needle attached to a raised saline reservoir. Retinal ischemia was induced by elevating the intraocular pressure to 120 mm Hg. Measurement of intraocular pressure was made by a TonoPen XL tonometer (Mentor, Norwell, MA) calibrated according to the manufacturer's instructions. A hand-held ophthalmoscope was used to visually inspect the retinal blood vessels and verify ischemia. After 45 min, the saline reservoir was lowered and intraocular pressure and retinal circulation were 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 (1 mg/kg; i.p.) with 17β-estradiol, DHEA-S, PRE-084 (σ_1 receptor agonist), BD1047 (σ_1 receptor antagonist) 30 min prior to the transient ischemic insult. After ischemia, rats were left to recover for 3 days (reperfusion). Three days after ischemia the animals were killed, the eyes were enucleated and the retinas were rapidly removed and frozen by immersion in liquid nitrogen for 10 min. The samples were then quickly powdered in a pre-cooled automatic apparatus (Microdismembrator, Broun), and the frozen powders were weighed. The following steps were carried out in a pre-cooled box at 0–5 °C, until a neutral perchlorate-free extract was obtained, which was then used for immediate enzymatic analysis (Drago et al., 2001) of lactate, glucose and ATP by using a spectrophotofluorimeter (Varian DMS 200). Each value was calculated from the mean of four measurements. Limit of sensitivity (+5%) was 5 ng for all substances assayed. Statistical comparisons were made by analysis of variance (ANOVA) for repeated measures and post hoc Dunnett's test for multiple comparison. A *P* value of 0.05 or less was considered as indicative of a significant difference.

3. Results

Compared to normal controls (no ischemia; CTR), animals with retinal ischemia showed a sustained increase in tissue lactate content (Fig. 1A). Other biochemical changes included decreased level of glucose and ATP

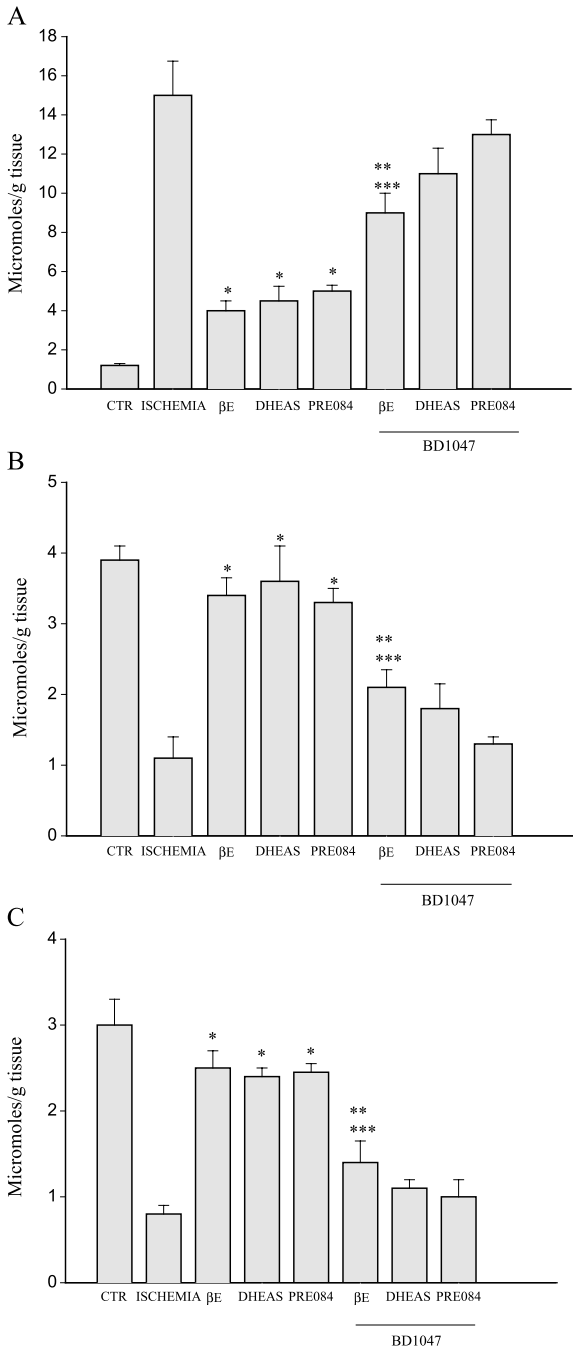

Fig. 1. Effects of 17β-estradiol (βE), DHEA-S and PRE-084 (1 mg/kg) against ischemia–reperfusion injury in the rat retina with or without BD1047 pre-treatment (1 mg/kg). (A) Levels of lactate, (B) glucose and (C) ATP in the retina samples after 3 days from ischemic damage. **P*<0.01 vs. ischemic group; ***P*<0.01 vs. βE alone; ****P*<0.05 vs. ischemic group. Each bar represents the mean±S.D. of four to six samples. CTR=normal controls (no ischemia).

(Fig. 1B and C). Treatments with the neurosteroids, 17β-estradiol or DHEA-S, prevented lactate accumulation in retinal tissue of rats subjected to ischemia, and induced an increase in glucose and ATP content. Similar results were obtained by treatment with the σ_1 receptor agonist, PRE-084 that prevented lactate accumulation in retinal tissue of animals subjected to ischemia by elevating ocular hypertension, and induced an increase in glucose and ATP content (Fig. 1A, B and C). In contrast, treatment with the σ_1 receptor antagonist, BD1047, failed to cause any biochemical change in retinal tissue both in normal controls (no ischemia) and in ischemic rats (data not shown). However, the effects of DHEA-S and PRE-084 were antagonized by pre-treatment with BD1047. Hence, the lactate content, the glucose and ATP levels in the DHEA-S and PRE-084 groups pre-treated with the BD1047 were comparable to the ischemic group (Fig. 1A, B and C). Furthermore, the protection exerted by 17β-estradiol was only partially antagonized by pre-treatment with BD1047.

4. Discussion

The present results show that 17β-estradiol and DHEA-S may improve the metabolic state of surviving neurons and glial cells after ischemic injury. These results seem to suggest that these drugs are neuroprotective although no evidence has been provided that neurosteroid administration influences the structural and functional deficits induced by ischemic injury to the retina. The effects described here appear to be due, at least in part, to involvement of the σ_1 recognition binding sites. In fact, PRE-084 is a potent inhibitor of radioligand binding to σ_1 site with K_i of 44 nM (Su et al., 1991), DHEA-S and 17-beta-estradiol also inhibited the binding to σ_1 sites with K_i values in the low micromolar range (Su et al., 1988).

Direct investigation of the protective effects of estrogen against ischemia–reperfusion-induced retinal damage demonstrated that 17β-estradiol reduces leukocyte accumulation and consequent retinal damage, particularly in the inner retina (Nonaka et al., 2000). More recently, administration of 17β-estradiol has been shown to protect the inner retina from apoptosis and early changes in synaptic connections associated with ischemia (Kaja et al., 2003). Kimodines et al. (1998) showed that DHEA and DHEA-S protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Furthermore, it has been demonstrated that DHEA-S has therapeutic potential in the prevention and treatment of ischemic–hypoxic neuronal damage (Kaasik et al., 2001). Despite these findings, the mechanism or the mechanisms by which neurosteroids exert these neuroprotective effects have not been completely elucidated. The evidence of a direct interaction between neurosteroids and σ_1 receptors was first suggested by the ability of several steroids to inhibit the binding of σ_1-receptor radioligands in vitro and in vivo (Maurice et al., 2001). A crossed pharmacology between neurosteroids and σ_1 receptors was described in various physiological tests and behavioral responses (Maurice et al., 2001). Several in vitro studies demonstrated that σ_1-receptor ligands exert neuroprotective properties (Nakazawa et al., 1998; Senda et al., 1998; De Loore et al., 1994). Senda et al. (1998) showed that σ_1-receptor agonists protect retinal cells against glutamate-induced neurotoxicity. The present study suggests, for the first time, that a possible protective effect of 17β-estradiol and DHEA-S on ischemia–reperfusion injury in the rat retina could be mediated by activation of σ_1 recognition sites. In fact, 17β-estradiol and DHEA-S were able to inhibit lactate accumulation and induced an increase in glucose and ATP content in retinal tissue, and these effects were partially or completely antagonized by a σ_1 receptor antagonist. However, we are aware that these effects cannot be considered dose-dependent as they were shown using only one dose of each neurosteroid. In conclusion, our findings support the hypothesis that there exists a crossed pharmacology between neurosteroids and σ_1 recognition sites, and that this may be of major importance in view of a therapeutic use of neurosteroids in retinal diseases.

Acknowledgements

This study was supported by a grant from M.I.U.R., Misura III.4 PON RST and AF 2000–2006 in a project of the PhD Program in Neuropharmacology, University of Catania.

References

De Loore, K., Lesage, A.S., Peeters, L., Leysen, J.E., 1994. Various σ ligands exert long-term protection against glutamate toxicity in primary hippocampal neurons; apparent non involvement of identified σ_2 sites. Neurosci. Res. Commun. 14, 43–51.

Drago, F., Valzelli, S., Emmi, I., Marino, A., Cassar Scalia, C., Marino, V., 2001. Latanoprost exerts neuroprotective activity in vitro and in vivo. Exp. Eye Res. 72, 479–486.

Hanner, M., Moebius, F.F., Flandorfer, A., Knaus, H.G., Streissneg, J., Kempner, E., Glossmann, H., 1996. Purification, molecular cloning and expression of the mammalian sigma1 binding site. Proc. Natl. Acad. Sci. U. S. A. 93, 8072–8077.

Kaasik, A., Kalda, K., Jaako, K., Zharkovsky, A., 2001. Dehydroepiandrosterone sulphate prevents oxygen-glucose deprivation-induced injury in cerebellar granule cell culture. Neuroscience 102, 427–432.

Kaja, S., Yang, S.H., Wei, J., Kazuko, F., Liu, R., Brun-Zinkernagel, A.M., Simpkins, J.W., Inokuchi, K., Koulen, P., 2003. Estrogen protects the inner retina from apoptosis and ischemia-induced loss of Vesl-1L/Homer 1c immunoreactive synaptic connections. Investig. Ophthalmol. Vis. Sci. 44, 3155–3162.

Kimonides, V.G., Khatibi, N.H., Svendsen, C.N., Sofroniew, M.V., Herbert, J., 1998. Dehydroepiandrosterone (DHEA) and DHEA-sulphate (DHEA-S) protect hippocampal neurons from excitatory amino acid-induced neurotoxicity. Proc. Natl. Acad. Sci. U. S. A. 95, 1852–1857.

Leitner, M.L., Hohmann, A.G., Patrick, S.L., Walker, J.M., 1994. Regional variation in the ratio of σ_1 to σ_2 binding in rat brain. Eur. J. Pharmacol. 259, 65–69.

Maurice, T., Urani, A., Phan, V.L., Romieu, P., 2001. The interaction between neuroactive steroids and the σ_1 receptor function: behavioural consequences and therapeutic opportunities. Brain Res. Rev. 37, 116–132.

Nakazawa, M., Matsuno, K., Mita, S., 1998. Activation of σ_1 receptor subtype leads to neuroprotection in the rat primary neuronal cultures. Neurochem. Int. 32, 337–343.

Nonaka, A., Kiryu, J., Tsujikawa, A., Yamashiro, K., Myamoto, K., Nishiwaki, H., Mandai, M., Honda, Y., Ogura, Y., 2000. Administration of 17β-estradiol attenuates retinal ischemia–reperfusion injury in rats. Investig. Ophthalmol. Vis. Sci. 41, 2689–2696.

Quiron, R., Bowen, W.D., Itzhak, Y., Junien, J.L., Musacchio, J.M., Rothman, R.B., Su, T.P., Tam, S.W., Taylor, D.P., 1992. A proposal for the classification of sigma binding sites. Trends Pharmacol. Sci. 13, 85–86.

Senda, T., Mita, S., Kaneda, K., Kikuchi, M., Akaike, A., 1998. Effect of SA4503, a novel σ_1 receptor agonist glutamate neurotoxicity in cultured rat retinal neurons. Eur. J. Pharmacol. 342, 105–111.

Su, T.P., London, E.D., Jaffe, J.H., 1988. Steroid binding at sigma receptors suggests a link between endocrine, nervous, and immune systems. Science 240, 219–221.

Su, T.P., Wu, X.Z., Cone, E.J., Shukla, K., Gund, T.M., Dodge, A.L., Parish, D.W., 1991. Sigma compounds derived from phencyclidine: identification of PRE-084, a new selective sigma ligand. J. Pharmacol. Exp. Ther. 259, 543–550.

Walker, J.M., Bowen, W.D., Walker, F.O., Matsumoto, R.R., De Costa, B.R., Rice, K.C., 1990. Sigma receptors: biology and function. Pharmacol. Rev. 42, 355–402.