Experimental Neurology 240 (2013) 146–156

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Regular Article

Post-ischemic environmental enrichment protects the retina from ischemic damage in adult rats

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ARTICLE INFO

Article history:
Received 1 August 2012
Revised 6 November 2012
Accepted 13 November 2012
Available online 27 November 2012

Keywords:
Retina
Ischemia
Enriched environment
Glutamate

ABSTRACT

The aim of this study was to elucidate whether post-ischemic enriched environment (EE) housing protects the retina from ischemic damage in adult rats, and the involvement of glutamate in retinal protection induced by EE housing. For this purpose, ischemia was induced by increasing intraocular pressure to 120 mm Hg for 40 min. After ischemia, animals were housed in a standard environment (SE) or EE and subjected to electroretinography and histological analysis. EE housing afforded significant functional protection in eyes exposed to ischemia/reperfusion injury. A marked reduction in retinal thickness and ganglion cell number, and an increase in Müller cell glial fibrillary acidic protein (GFAP) levels were observed in ischemic retinas from SE-housed animals, which were reversed by EE housing. A deficit in anterograde transport from the retina to the superior colliculus was observed in SE- but not in EE-housed animals. In SE-housed animals, ischemia induced a significant decrease in retinal glutamate uptake and glutamine synthetase activity, whereas EE housing reversed the effect of ischemia on these parameters. The intravitreal injection of supraphysiological levels of glutamate partially reproduced retinal alterations induced by ischemia/reperfusion, which were abrogated by EE housing. These results indicate that EE housing significantly protected retinal function and histology from ischemia/reperfusion injury in adult rats, likely through a glutamate-dependent mechanism.

Introduction

Ischemia is one of the key factors determining the pathophysiology of many retinal diseases, such as diabetic retinopathy, glaucoma, and age-related macular degeneration, among others. Retinal hypoxia and ischemia impair neuronal energy metabolism by launching a cascade of trigger reactions resulting in cell death. In addition, reperfusion with oxygenated blood after ischemia aggravates ischemic damage, an effect known as reperfusion injury. Several mechanisms such as excitotoxicity, oxidative stress, cell acidosis, and inflammation, acting in tandem are of considerable importance in retinal ischemia (reviewed by Osborne et al., 2004). Glutamate is the main excitatory neurotransmitter in the retina but it is toxic when present in excessive amounts. Thus, effective buffering of extracellular glutamate is important in preserving retinal structure and function. Glutamate excess is thought to cause neuronal cell death in the retina during ischemia/reperfusion (I/R) (Osborne et al., 2004). It was shown that electrophysiological and neuronal damage following ischemia resembles that caused by exposure to supraphysiological levels of glutamate (Fernandez et al., 2009a; Ikeda et al., 1992; Iversen, 1991) and that retinal ischemia induces a significant increase in glutamate release (Cazevieille and Osborne, 1997; Neal et al., 1994). At present, there is no effective treatment to protect the retina from I/R damage. Therefore, the development of resources to protect the retina against ischemia is a goal of vast clinical importance.

Besides pharmacological treatments, environmental conditions have been shown to modify the extent of ischemic damage in the central nervous system (Belayev et al., 2003; Briones et al., 2000; Ohlsson and Johansson, 1995). Enriched environment (EE) refers to conditions that facilitate or enhance sensory, cognitive, motor, and social stimulation relative to standard (laboratory) conditions. Several studies demonstrated that animals housed in EE after ischemic stroke obtain a better functional and structural outcome as compared with those housed in standard cages (Biernaskie and Corbett, 2001; Nygren and Wieloch, 2005; Ohlsson and Johansson, 1995; Sun et al., 2010). Moreover, it has been shown that EE causes several morphological and neurochemical changes in the central nervous system, like thicker cortex, increased dendritic spine density, increased expression of neurotrophic factors, and enhanced neurogenesis (Franklin et al., 2006; Nithianantharajah and Hannan, 2006).

Abbreviations: CTB, cholera toxin β-subunit; EE, enriched environment; I/R, ischemia/reperfusion; ERG, electroretinogram; OPs, oscillatory potentials; GFAP, glial fibrillary acidic protein; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OS, photoreceptor outer segments; RGCs, retinal ganglion cells; SC, superior colliculus; SE, standard environment.

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http://dx.doi.org/10.1016/j.expneurol.2012.11.017

Retinal development is responsive to the experience provided by EE: the maturation of retinal acuity, which is a sensitive index of retinal circuitry development, is strongly accelerated by EE in rats (Landi et al., 2007). Prusky and co-workers have demonstrated that mice exposed to an EE from birth have an enhanced visual acuity when compared to restrained controls, suggesting that the performance of the visual system is greatly influenced by the complexity of the visual environment (Prusky et al., 2000). In addition, it was reported that EE provides protection against retinal degeneration induced by neonatal glutamate treatment in rats (Szabadfi et al., 2009). In contrast, the adult retina has long been considered less plastic than the brain cortex or hippocampus, the very sites of experience-dependent plasticity, and until now less is known on the protective effects of EE housing in the retina from adult rats. In that context, the aim of the present report was to analyze the beneficial effect of post-ischemic environmental enrichment on I/R damage in adult rats.

Materials and methods

Animals

All animal use procedures were in strict accordance with the NIH Guide for Care and Use of Laboratory Animals. The ethics committee of the University of Buenos Aires School of Medicine, (Institutional Committee for the Care and Use of Laboratory Animals, (CICUAL)) approved this study. Adult male *Wistar* rats (average weight, 250 ± 50 g) were housed in a standard animal room with food and water *ad libitum*, under controlled conditions of humidity and temperature (21 ± 2 °C). The room was lighted by fluorescent lights (200 lx) that were turned on and off automatically every 12 h (on from 8.00 AM to 8.00 PM). Animals from the control group (standard environment, SE) were housed in standard laboratory cages ($33.5 \times 45 \times 21.5$ cm) with two animals per cage. For enriched environment (EE) housing, six animals at a time were housed in big cages ($46.5 \times 78 \times 95$ cm), containing four floors and several food hoppers, water bottles, running wheels, tubes, ramps and differently shaped objects (balls, ropes, stones) repositioned once a day and fully substituted once a week. Particular care was taken not to repeat cage arrangement and object availability during the experiments. Animals were caged in the EE immediately after ischemia. Although food and water were offered *ad libitum*, location of the hoppers and bottles was changed daily in order to stimulate exploratory conduct. Cages were cleaned once a week at the same time and by the same protocol to that used for standard cage cleaning. The body weight was weekly monitored, and no significant differences in this parameter were observed between animals housed in SE and EE. Experimental groups and the experimental design used in this study are depicted in Fig. 1.

Retinal ischemia

Animals were anesthetized with ketamine hydrochloride (150 mg/kg) and xylazine hydrochloride (2 mg/kg) administered intraperitoneally. After topical instillation of proparacaine, the anterior chamber of each eye was cannulated with a 30-gauge needle connected to a pressurized bottle filled with sterile normal saline solution. Retinal ischemia was induced in one eye by increasing intraocular pressure to 120 mm Hg for exactly 40 min, as previously described (Fernandez et al., 2009a). With this maneuver, complete ocular ischemia was produced, characterized by cessation of flow in retinal vessels, determined by funduscopic examination. During and after (before animals were returned to the animal house) the experiments, animals were kept normothermic with heated blankets. The contralateral eye was submitted to a sham procedure (*i.e.* eyes were cannulated without raising IOP); this procedure did not affect retinal function and histology as compared to intact eyes. A few animals in which cataracts developed due to lens injury, were not used any further in the experiments.

Electroretinography

Electroretinographic activity was assessed at 1, 2, and 3 weeks after ischemia, as well as at 7 days after an intravitreal injection of vehicle or glutamate, as previously described (Fernandez et al., 2009a). Briefly, after 6 h of dark adaptation, rats were anesthetized under dim red illumination. Phenylephrine hydrochloride and tropicamide were

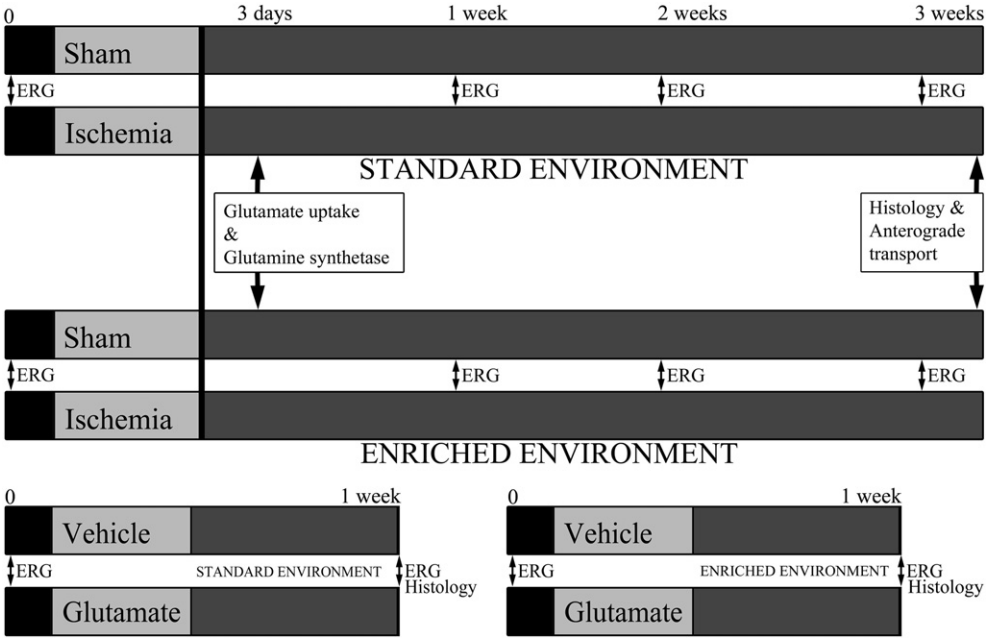

Fig. 1. Experimental groups. Animals were submitted to retinal ischemia, and caged in SE or EE immediately after ischemia. Electroretinograms were recorded at 1, 2, and 3 weeks after ischemia. Glutamate uptake and glutamine synthetase activity were assessed at 3 days post-ischemia, and histological and anterograde transport studies were performed at 3 weeks after ischemia. In another set of experiments, eyes were intravitreally injected with vehicle or glutamate, and animals were housed in SE or EE. In this case, functional and histological studies were performed at 1 week post-injection.