Neuroscience Letters 469 (2010) 84–87

Contents lists available at ScienceDirect

Neuroscience Letters

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

Effects of aging and every-other-day feeding on the levels of oxygen radicals in rat brain slices

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ARTICLE INFO

Article history:
Received 15 October 2009
Received in revised form
16 November 2009
Accepted 18 November 2009

Keywords:
Aging
Reactive oxygen species
Every-other-day feeding
Brain slices
Hypoxia-reoxygenation

ABSTRACT

Caloric and food restriction attenuate oxidative stress. The effect of aging and every-other-day (EOD) feeding on oxygen radical-dependent chemiluminescent intensity was examined in ex vivo brain slices from Fischer rats during oxygenation and hypoxia-reoxygenation with lucigenin, a chemilumigenic probe used for detecting superoxide anion radicals. The chemiluminescent intensity increased during reoxygenation after hypoxic treatment, and the chemiluminescence in the brain slices at the baseline and during reoxygenation increased with age. However, no difference was observed in the superoxide-dependent chemiluminescence between brain slices prepared from the aged rats fed EOD and those fed ad libitum. Our results indicated that age-dependent increases in superoxide production might be associated with enhanced oxidative stress in aged Fischer rat brains. However, the present study newly indicated that decreased superoxide production might not be a major causal factor in caloric and food restriction attenuated oxidative stress.

Caloric and food restriction extends the maximum and mean life spans of animals including yeast, houseflies, nematodes, fishes, spiders, hamsters, mice, rats, dogs, and nonhuman primates [2,10,13,15,16,20]. It is also effective at preventing ischemia [30], Parkinson's disease [3], Alzheimer's disease [17,31], and carcinogenesis [8,29]. Oxidative stress is believed to be a major causal factor in the aging process [7], and age-related increases in markers of oxidative damage are known to be delayed by caloric and dietary restriction [1,5,9,12,18,21,25–27].

Previously, we have demonstrated that the superoxide-dependent chemiluminescent intensity in animal brain slices increases linearly with age [24]. Here, we examined whether the superoxide-dependent chemiluminescent intensity observed in Fischer rat brain slices during oxygenation and hypoxia-reoxygenation increases with age and whether it is delayed by every-other-day (EOD) feeding compared with that in ad libitum fed rats.

The animals were sacrificed by decapitation under light diethyl ether anesthesia, and their brains were rapidly removed and placed on a tissue cutter (Microslicer DTK-3000W; Dosaka EM, Kyoto, Japan). Coronal slices cut 300-μm thick were transferred into ice-cold Krebs-Ringer solution (124 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.2 mM KH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose) equilibrated with 95% O_2/5% CO_2. The imaging chamber was filled with 50 mL of Krebs-Ringer solution containing 2 mM of N,N′-dimethyl-9,9′-biacridinium dinitrate (lucigenin) and was placed in a temperature-controlled dark box at 34 °C. A continuous flow of gas (100 mL 95% O_2/5% CO_2/min/one chamber) was delivered into the solution by bubbling. Before further treatment, eight brain slices from each rat were preincubated for 45 min in the chamber.

The acquisition of superoxide-dependent chemiluminescence images of brain slices during oxygenation and hypoxia-reoxygenation, a model of ischemia-reperfusion, was performed using a novel photonic imaging method, "real-time bioradiography" [23,24]. After a 45-min preincubation period, the slices were incubated for an additional 120 min in the same oxygenated environment (95% O_2/5% CO_2) in an imaging chamber at 34 °C. Then, further incubation under hypoxic (95% N_2/5% CO_2) conditions was performed for 15 min, before a return to an oxygenated environment for up to 120 min. Images of the brain slices were acquired every 15 min during the oxygenated, hypoxic, and re-oxygenated phases for up to 255 min (17 frames). The images were acquired under a 9 cm × 12 cm field-of-view.

A region of interest (ROI) was selected from the whole brain slices. Chemiluminescent emission was expressed as "counts/pixel/min", which represents the chemiluminescent emission per unit area in 15 min. The steady-state level of chemiluminescent emission under oxygenated conditions (baseline) was expressed as "counts/pixel/min", which was calculated by averaging the chemiluminescent intensity of eight brain slices from

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doi:10.1016/j.neulet.2009.11.049

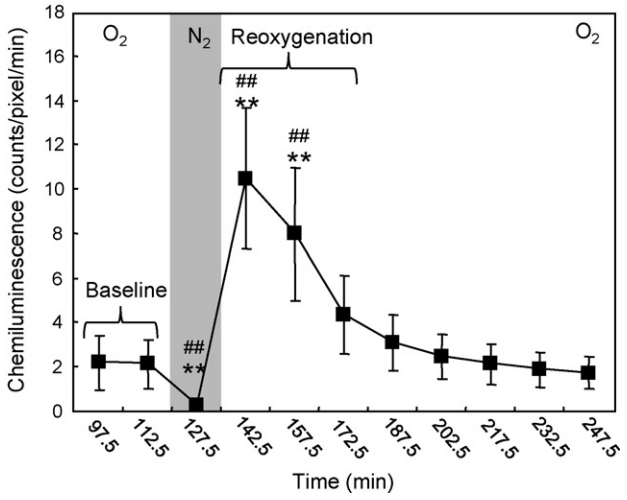

Fig. 1. Typical pattern of the changes in chemiluminescent intensity in Fischer344/DuCrj rat (3 months old) brain slices during oxygenation and hypoxia-reoxygenation. Images of eight brain slices from each rat were acquired every 15 min during the oxygenated, hypoxic, and re-oxygenated phases for up to 255 min (17 frames). Superoxide-dependent chemiluminescent intensity expressed as "counts/pixel/min" is represented as the mean ± SD in each frame. Statistical significance was determined by ANOVA and Tukey-HSD test ($^{**}P < 0.01$ from the 7th frame (90–105 min) and $^{\#\#}P < 0.01$ from the 8th frame (105–120 min) value). The steady-state level of chemiluminescent emission under oxygenated conditions (baseline) was calculated for further experiments by averaging the chemiluminescent intensity during the 30 min prior to hypoxic treatment and that for reoxygenation (reoxygenation) was calculated during the 45 min post-hypoxic treatment.

each rat during the 30 min prior to hypoxic treatment, and that for reoxygenation (reoxygenation) was calculated during the 45 min post-hypoxic treatment as shown in Fig. 1.

Three-week-old male Fischer 344 rats were purchased from Charles River Japan (Kanagawa, Japan) and were kept in the barrier animal facility of the Tokyo Metropolitan Institute of Gerontology and at a density of 3 rats per plastic cage under conventional conditions with a constant temperature and humidity and a 12 h light–dark cycle. The rats were fed a commercial laboratory chow diet (CRF-1: Oriental Yeast Co. Ltd., Tokyo, Japan). Rats of 1, 3, 10, 22, and 28 months old were used for this experiment. Chemiluminescent images were acquired during oxygenation and hypoxia-reoxygenation of the eight brain slices prepared from each rat, and the levels of chemiluminescent emission at the baseline and under reoxygenation conditions were analyzed in the same manner as described above. The values obtained at the baseline and under reoxygenation were averaged and represented as the mean ± standard deviation for 1-($n = 7$), 3-($n = 4$), 10-($n = 5$), 22-($n = 6$), and 28-month-old ($n = 8$) rats.

More 3-week-old male Fischer 344 rats were purchased from Charles River Japan and kept in the barrier animal facility of our institute under the same conditions as described above. One group of animals was fed ad libitum, and the other was fed restrictively on a commercial laboratory chow diet (CRF-1). EOD feeding was carried out using an intermittent feeding method, and the animals were fed 3 days a week (Monday, Wednesday, and Friday). The rats were subjected to two different feeding regimes, ad libitum and EOD feeding, until sacrifice. Pairs of ad libitum and EOD fed 22- and 28-month-old rats were used for this experiment. Chemiluminescent images were acquired during oxygenation and hypoxia-reoxygenation in brain slices, and the levels of chemiluminescent emission at the baseline and under reoxygenation were analyzed as described above. The values observed at the baseline and under reoxygenation were averaged and represented as the mean ± standard deviation of the 22-($n = 4$) and 28-month-old ($n = 4$) ad libitum rats and the 22-($n = 4$) and 28-month-old EOD fed rats. The mean food intake per week of the EOD fed rats was about 50–60% of that of the ad libitum fed rats at all ages, and the body weight of the EOD fed rats increased more slowly than that of the ad libitum fed rats. At 22- and 28 months old, the mean body weight of the dietary-restriction group was about 45–55% of that of the ad

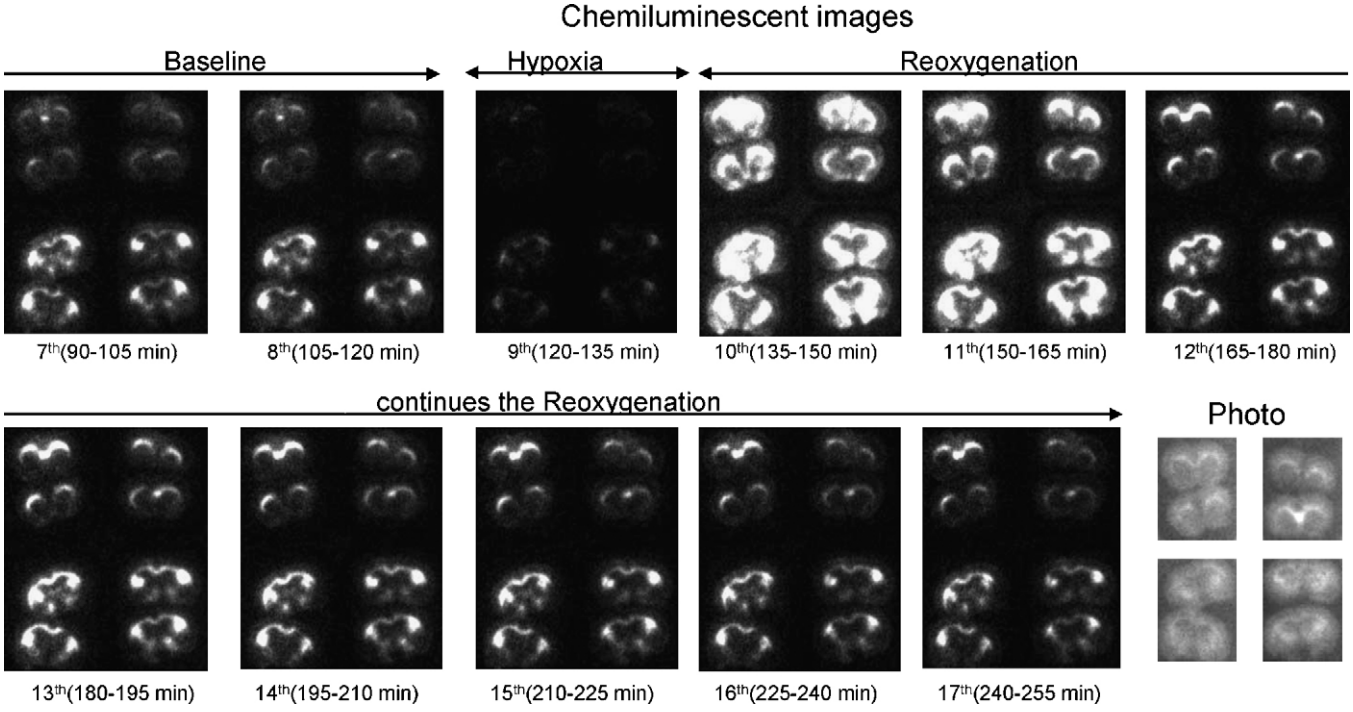

Fig. 2. Typical chemiluminescent images in Fischer344/DuCrj rat (3 months old) brain slices during oxygenation and hypoxia-reoxygenation. The images were acquired from the 1st frame (45 min after preincubation) to the 17th frame and are shown here from the 7th frame onwards. Image brightness in each frame is represented by the same scale.