Formation of Lipoperoxide in the Retina of Rabbit Exposed to High Concentration of Oxygen

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Summary. When rabbit was exposed to high concentration of oxygen, lipoperoxide in the retina was increased at 12 h of the exposure, after which period amplitude of electro-retinogram decreased. The degeneration was observed in the visual cell layer of the retina of the exposed animal. The exposure increased lipoperoxide in isolated retina. These data show the intervention of lipoperoxide in retinal degeneration induced by exposure to high concentration of oxygen.

It is well known that the retina is one of the tissues which are most susceptible to oxygen. NOELL[2,3] reported that exposure of adult rabbits to high concentration of oxygen at ambient pressure for a few days induces retinal degeneration which is similar to that induced by X-ray irradiation. NOELL's work was based on the earlier results of GERSCHMAN et al.[4], who reported that irradiation and oxygen poisoning produce their lethal effects through a common mechanism, possibly through the formation of oxidizing free radicals. Several workers have reported that X-ray irradiation induces peroxidation of lipids[5–13]. Thus, as pointed out by HAUGAARD[14], peroxidation may play a role in the toxic effects of high concentrations of oxygen. In fact, it has been reported that lipoperoxide level increases in the brain of animals exposed to elevated pressures of oxygen[15,16]. On the basis of these reports, we decided to see if lipoperoxides are produced in the retina of rabbit during oxygen-mediated degeneration of the retina.

Animals used were adult albino rabbits weighing 2–3 kg; the animals were placed in the chamber, in which oxygen concentration was maintained between 90% and 95% at ambient pressure. Accumulation of carbon dioxide was minimized by a small continuous flow of oxygen through the chamber. Food and water were supplied to the animals during the experiments. Temperature in the chamber was controlled at 20–25 °C. The control animals were placed in the chamber which was flowed by air in the same way as above.

After the animals were exposed to oxygen for different periods, the electro-retinogram (ERG) amplitudes were measured. After 20 min of dark adaptation, ERG was recorded with time constant, 0.03 sec, using xenon light stimulus of 2.0 jouls. Lipoperoxide in the retina was determined by thiobarbituric acid (TBA) method[17]. The procedure is similar to that reported by NISHIGAKI et al.[18]. The eyes of an animal were enucleated and the retinas were taken for each measurement. After recording wet weight, the retinal tissues were homogenized in 0.5 ml of cold 0.9% NaCl aqueous solution. The homogenate was transferred to a centrifuge tube and made up to 2.0 ml with 0.9% NaCl. To the homogenate, 5.0 ml of TBA reagent (the mixture of equal volumes of 0.67% TBA aqueous solution and glacial acetic acid) were added, and heated at 95 °C in an oil bath for 1 h. After cooling with tap water, 5.0 ml of chloroform were added, shaken vigorously and centrifuged at 3,000 *g* for 10 min. The supernatant was centrifuged further at 10,000 *g* for 10 min, and clear supernatant obtained was subjected to absorbance measurement at 532 nm.

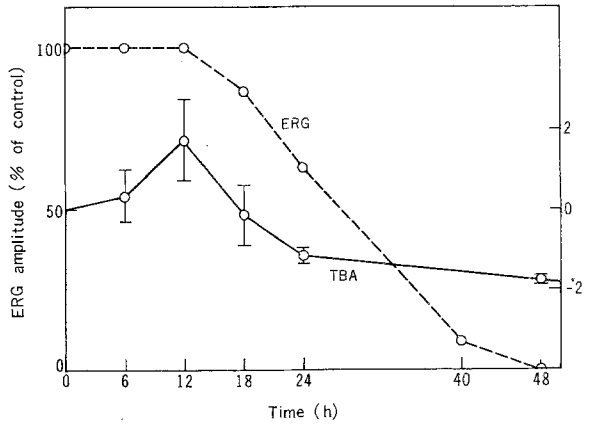

Fig. 1. Changes in ERG and TBA value in the retina of rabbit upon exposure to high concentration of oxygen. For experimental conditions, see text. The amplitudes of ERG (a- and b-waves) were represented by percent of mathematical means, obtained by multiple measurements for at least 4 animals exposed to oxygen, to those of the controls. TBA values were represented by mathematical means of the difference between the data obtained for at least 4 animals with and without oxygen exposure. Bars represent standard deviation calculated from the difference (σ_D).

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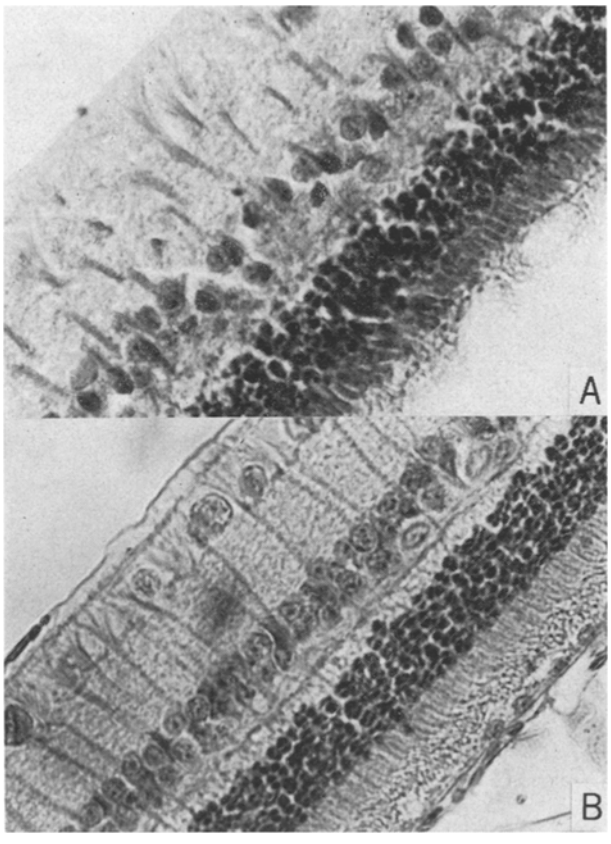

Fig. 2. Light microscopic observation on the retina of rabbit exposed to high concentration of oxygen for 40 h (A) and that of the control (B).

As shown in Figure 1, the exposure to oxygen for 12 h resulted in the increase in lipoperoxide in the retina as compared with the control ($p < 0.02$). Upon continuing the exposure to oxygen, the content of lipoperoxide began to decrease and reached a level lower than that of the control (at 24 h, $p < 0.01$; at 48 h, $p < 0.001$). After the exposure to oxygen for 12 h, the ERG amplitude began to decrease and became non-recordable after the exposure for 48 h (Figure 1). It should be noted that ERG began to decrease in accordance with the increase in lipoperoxide.

To observe the histological change in the retinal tissue, a light microscopic observation was made. Figure 2A shows the microphotograph of the retina of rabbit exposed to oxygen for 40 h. Comparison with the control (Figure 2B) obviously indicates pronounced degeneration in the visual cell layers in the case of oxygen exposure.

To measure the lipoperoxide formation in isolated retinas, the following experiments were carried out. Retinas were suspended in 0.9% NaCl and saturated with pure oxygen flowing from a pipette into the test tube for 45 min. The control experiment was done with argon flowing under the same conditions. These experiments were performed at room temperature and at ambient pressure. Lipoperoxide formation of retina suspension was measured by the above-mentioned TBA reaction. The amount of lipoperoxide in isolated retina exposed to oxygen increased approximately $2^1/_2$ times as compared with that of the control.

These results suggest the possibility that the formation of lipoperoxide in the retina is induced by high concentration of oxygen and the lipoperoxide denatures the associated proteins, resulting in an inability of retinal function as observed by ERG, and the change in structure as observed by light microscope.

Myopathic Changes at the End-Plate Region Induced by Neostigmine Methylsulfate

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Summary. Administration of large dose of neostigmine caused very quickly marked myopathic changes at the motor end-plate region. With continued injections, however, some recovery of the structural features did occur suggesting the reconstructive changes in the affected regions.

Neostigmine is a reversible anticholinesterase drug for the treatment of myasthenia gravis and other diseases. Previous studies have indicated that the repeated administration of this drug can produce some changes at the motor end-plates, such as atrophy of post-synaptic folds and the appearance of collapsed residues of pre-existing folds in widened synaptic spaces[3,4]. We report here that the repeated administration of large amounts of neostigmine methylsulfate produces not only changes at the motor end-plates, but also striking changes in the areas of muscle fibres adjacent to the motor end-plates.

Materials and methods. A total of 44 Sprague-Dawley rats weighing 200–250 g were used. 2 rats were injected s.c. with neostigmine methylsulfate at a dose of 0.06 mg/kg, 0.1 mg/kg, 0.25 mg/kg and 0.625 mg/kg (resolved in 1 ml saline solution), respectively. They were sacrificed 2 h after the injection. 10 rats received a single dose of 0.625 mg/kg and were sacrificed after 1 week and 2 weeks. 15 rats were given a daily injection with a dose of 0.625 mg/kg and sacrificed after 3 days, 1 week, 3 weeks, 6 weeks and 8 weeks.

In 6 other rats the right sciatic nerve was sectioned. From the 6th postoperative day, daily injections with a dose of 0.625 mg/kg were started and the rats were sacrificed after 2 days, 1 week and 3 weeks. 5 control rats were injected with equal volumes of physiological saline solution daily for 3 weeks and then sacrificed. Within 5 to 10 min following the injection, all of the

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