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DAY–NIGHT CYCLE OF LIPID PEROXIDATION IN RAT CEREBRAL CORTEX AND THEIR RELATIONSHIP TO THE GLUTATHIONE CYCLE AND SUPEROXIDE DISMUTASE ACTIVITY

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Abstract—Lipoperoxidation, glutathione cycle components and superoxide dismutase activity show a day–night rhythm in the cerebral cortex of the rat. The highest lipoperoxidative activity is observed during the night (20.00–04.00 h). The enhancement in lipoperoxidation occurs concurrently with a decrease in glutathione peroxidase activity, an increase in superoxide dismutase activity and an increase in the double bonds in the brain cortex lipid fraction. The changes described in this paper seem to be related to a succession of light and dark periods, or to fasting and feeding periods.

We propose that those fluctuations could act as a physiological oscillator with an important role in modulating the membrane properties of the nerve cell.

Lipoperoxidation has been considered a damaging reaction in biological systems usually associated with aging[16] and degenerative processes.[28] Peroxidation of phospholipid unsaturated fatty acids is accompanied by structural and functional changes of membranes.[6,10] These changes have been correlated with certain physiological roles of cellular activity such as enzyme activation,[1] inactivation[4] and modifications in some membrane properties such as fluidity, permeability and surface potential.[5]

The brain is an organ with the highest rate of lipoperoxidation,[32] probably because of its high oxygen uptake and high unsaturated fatty acid concentration as phospholipid constituents. In the brain, regional differences in lipid peroxidation which have been observed[26] could result from an unequal production of endogenous free radicals, from different antioxidant activities, mainly of glutathione peroxidase (GSH-per) and of superoxide dismutase (SOD), or from an unequal proportion of unsaturated fatty acids.

Recently, we reported an antilipoperoxidative action of adenosine in liver damage induced by carbon tetrachloride.[19] We also reported day–night variations of adenosine in the liver, blood and brain.[8,9] These observations plus the possible role of adenosine as a neuromodulator,[14,15,29] suggested the possibility that the nucleoside was involved in the lipoperoxidation activity of the brain.

We therefore looked for day–night changes in brain lipoperoxidation, specifically in the cerebral cortex, which is one of the brain regions with the largest lipoperoxidative activity.[26] We also investigated the participation of the glutathione cycle and the superoxide dismutase activity in the lipoperoxidative process.

EXPERIMENTAL PROCEDURES

Male Wistar rats weighing 160–180 g, with free access to food and water were used. The animals were kept in groups of seven in separate cages and adapted to a dark–light cycle (the 12 h of light were from 07.00 to 19.00) for at least two weeks before the experiment; on the day of the study, maximum precautions were taken to avoid stress. Animals were killed at 4 h intervals from 08.00 to 20.00 h and every 2 h from 22.00 to 07.00 h.

All the studies were made in the cerebral cortex. Lipid peroxidation was measured in a 1:10 bidistilled water homogenate, by the thiobarbituric acid method,[27] modified as previously reported.[19] Glutathione was measured by an enzymatic method.[22] Glutathione peroxidase (EC 1.11.1.9) and glutathione reductase (EC 1.6.4.2) (GSH-R) were assayed according to the method of Sies[30] and Horn,[20] respectively. Superoxide dismutase (EC 1.15.1.1) (SOD) was measured according to McCord and Fridovich[24] and the tissue was handled according to Ledig.[23] In order to determine the amount of unsaturated linkages on lipids, these were extracted by the Folch method,[13] and quantified by the Wijs method[33] as follows: the lipid extract obtained after the Folch extraction was suspended in 5 ml of chloroform, 10 ml of Wijs's solution (iodine monochloride) were added and the mixture was maintained in the dark for 30 min. The iodine excess is titrated in the presence of 8 ml of 10% KI with 0.1 N sodium thiosulfate. When the color has practically disappeared, 1 ml of a 1% starch solution is added and the titration is continued until the blue color disappears. A blank with no lipid extract is substracted from the above values. Protein was determined by the biuret method.[21]

The statistical significance of the results was calculated by Student's *t*-test.

Materials

2-Thiobarbituric acid, methylglyoxal, egg albumin, sodium azide, reduced and oxidized glutathione, nicotinamide

Abbreviations: GSH, reduced glutathione; GSH-per, glutathione peroxidase; GSH-R, glutathione reductase; SOD, superoxide dismutase.

adenine dinucleotide phosphate (reduced form) and horse cytochrome Type III were purchased from Sigma Chemica Co. (St Louis, Missouri) as well as the following enzymes: glyoxalase I, glutathione reductase and xanthine oxidase. Perhydrol was obtained from Merck; and xanthine was purchased from Nutritional Biochemical Corp. (Cleveland, Ohio). Wijs solution was obtained from Sigma of México. Other chemicals used were reagent grade.

RESULTS

Lipid peroxidation in cerebral cortex changes markedly during the 24 h. The level of lipid peroxidation in the morning (08.00–12.00 h) is low and similar to that reported by Noda[26] (Fig. 1). At the onset of the dark period, peroxidation starts to increase and becomes significantly elevated at 20.00 h. This high level (100% increase) of lipid peroxidation is maintained for 8 h during the night (20.00–04.00 h); at this point the process initiates its decline reaching its lowest value at 08.00 h.

We can consider three possible explanations for this result: (a) a decrease in activity of a system that removes hydroperoxides; (b) an increase in peroxide concentration, and (c) an increase in polyunsaturated fatty acids as possible substrates of lipoperoxidative activity.

The first possibility was explored by measuring day–night variations of some of the parameters of the glutathione cycle such as the levels of GSH, GSH-per, GSH-R. The results are shown in Fig. 2. Fluctuations of GSH concentrations (panel A) were inversely related to lipoperoxidation, as reported by Ahmed,[11] with a maximum (2 μmol g^{-1}) from 12.00 to 22.00 h, and a minimum (1 μmol g^{-1}) at 02.00 h. The activity of GSH-per (panel B) shows a similar pattern to that of GSH, except at 22.00 h. The GSH-R profile (panel C) is different from the others; its activity remains constant during the day except for a peak with an almost fivefold increase in activity at 20.00 h. These results indicate that the activity of the glutathione system is low during the night when lipoperoxidation is high.

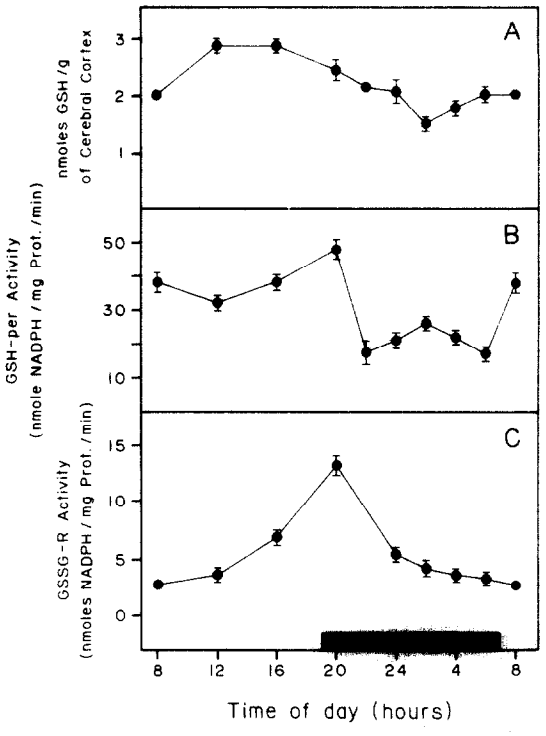

Fig. 2. Day–night changes of some components of the glutathione cycle in the cerebral cortex of the rat brain. The values represent the mean ± SE of five samples. The abscissa indicates the time of the day and the black region represents the dark period. (A) Reduced glutathione; (B) glutathione peroxidase activity; (C) glutathione reductase activity. Both of them represented as a decrease of NADPH, nicotinamide adenine dinucleotide phosphate, reduced.

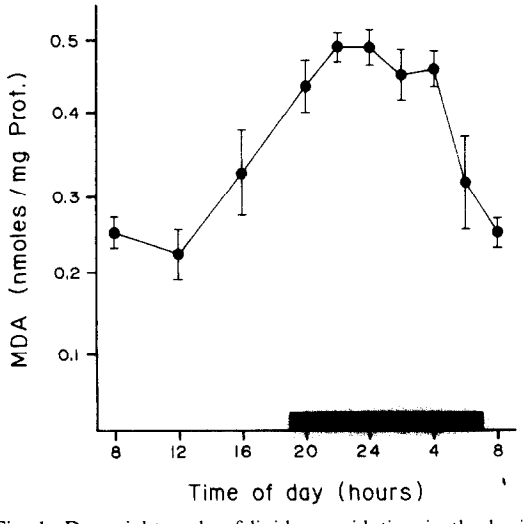

Fig. 1. Day night cycle of lipid peroxidation in the brain cortex of the rat. Malondialdehyde (MDA) was measured by the thiobarbituric acid method. Each value represents the mean ± SE of 14–16 animals. The abscissae indicate the time of the day and the black region correspond to the dark period.

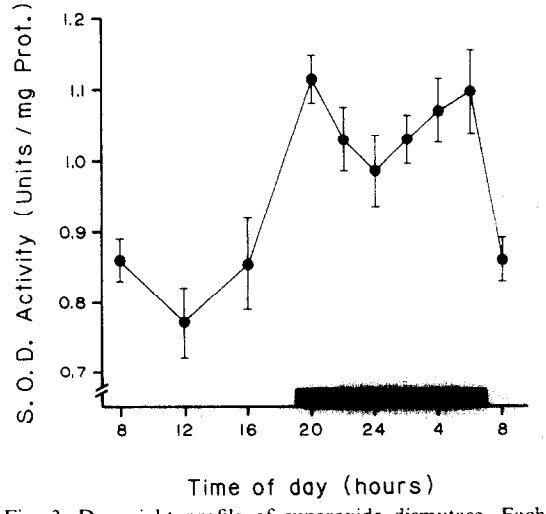

Fig. 3. Day–night profile of superoxide dismutase. Each value represents the mean ± SE of five samples. The abscissa indicates the time of the day and the black region corresponds to the dark period.

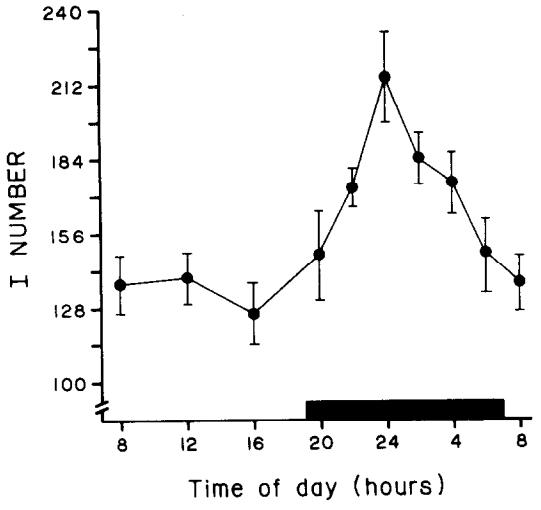

Fig. 4. Day–night cycle of the number of unsaturated bonds (I) in lipids extracted from the brain cortex. They were calculated by the Wijs method. The values represent the mean ± SE of five determinations. The abscissa indicate the time of the day and the black region corresponds to the dark period.

The second possibility, an increase in peroxide concentration, is feasible since a lowering of GSH-per activity, one of the most important enzymes encharged with peroxide removal, was observed (Fig. 2A); the production of peroxides was also followed through the superoxide dismutase reaction for the 24 h period. The results are shown in Fig. 3; changes in SOD are similar to that of lipoperoxidation (Fig. 1): low during the light period (08.00–16.00 h) and markedly increased during the dark (20.00–06.00 h). These results indicated that there is a major production of peroxides through the SOD reaction during the night.

The third possibility was explored by quantifying the unsaturation of the lipid extract of the brain cortex and determining the iodine number. The results are presented in Fig. 4. The number of double bonds remains low during the light period (08.00–16.00 h), and then starts to increase, reaching a peak at 24.00 h (210 I number). This shows that there is an increase in polyunsaturated fatty acids during the night.

DISCUSSION

Our results showed day–night variations of lipoperoxidation in the cerebral cortex and a corresponding fluctuation of GSH concentration, one of the natural lipid peroxidation inhibitors; GSH-R, GSH-per and SOD, all of them enzymes involved in the antioxidant systems of the cell, also showed fluctuations.

The high concentration of readily oxidizable compounds in the brain and the high oxygen consumption indicate the requirement for an efficient mechanism to prevent reactions of some active species of oxygen. During the light period the glutathione cycle works efficiently (Fig. 2); there is a high level of glutathione that is in an inverse relation to lipid peroxidation, as was noted by other authors.[11] Possibly, there is a high turnover rate for GSH since there is also an increase in the activity of the GSH-forming enzyme (GSH-R); GSH-per activity that used GSH to remove hydroperoxides, is also increased. At the onset of the dark period and the increase in lipid peroxidation, a decrease in the activity of the glutathione systems was observed; this suggests that a decrease in one of the main systems of removal of hydroperoxides could be partly responsible for the high night lipoperoxidation.

The role of superoxide ions (O_2^-) in the nocturnal increase of rat brain cortex lipoperoxidation is evident, since the variations of SOD activity parallel the changes in lipoperoxidation. Although the causes for SOD activity induction are not clear,[12] an increase in superoxide ion concentration could, among other factors, enhance the cytosolic SOD activity.[17] Rat motor activity and its feeding period occur in the dark; it is possible that an increase in superoxide ions and SOD activity induction might occur as a consequence to the metabolic burst.

On the other hand, adenosine has been postulated as a physiological modulator of superoxide anion generation in neutrophils;[7] it could possibly play a similar role in the brain since the day–night variations of brain adenosine seen in our laboratory[8,9] indicate a high brain adenosine level when SOD activity is low (Fig. 3); as soon as the nucleoside decreases, the activity of SOD increases. The results shown in Fig. 3, indicate that a major production of perioxides via a superoxide dismutation reaction may also be involved in the increase in lipoperoxidation during the night.

A pertinent observation is that simultaneously to a decrease in the glutathione cycle and an increase in SOD activity, there is also an increase in the number of unsaturated bonds of cerebral cortex lipids (Fig. 4), probably related to an increase in polyunsaturated fatty acids from the diet. It has been reported that triglycerides and free fatty acids constitute a low percentage of the total lipids of the brain;[31] the increase in the number of unsaturated double bonds might correspond to the fatty acids of the membrane phospholipids or to other lipids of the brain cortex membrane.

We do not know the sequence of events that participate in the day–night variations of lipoperoxidation, but it seems likely that a decrease in the hydroperoxide-removing system, an increase in the hydroperoxides due to SOD activity and an increase in the substrates for lipoperoxidative activity are involved (Fig. 5). It is possible that during the night there is an accumulation of H_2O_2, resulting from GSH-per inhibition and SOD stimulation, but we can not comment on this since we have not yet studied H_2O_2 fluctuations through the 24 h cycle.

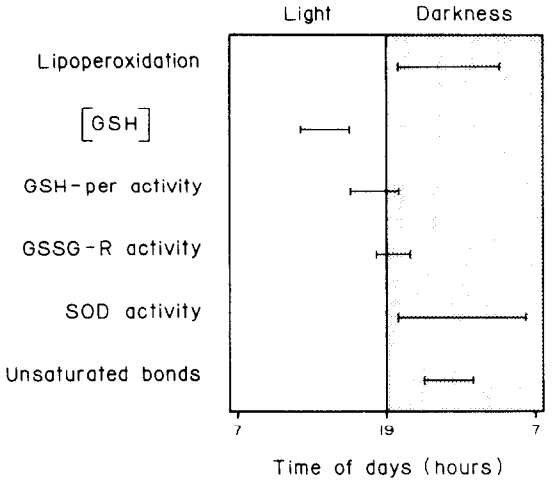

Fig. 5. Time relationship of the length of maximal activities of lipoperoxidation parameters studied in the cerebral cortex of the rat.

After this study was finished, a report of day–night variations of GSH in relation to lipid peroxidation in several rat tissues was published.[11] No significant changes were found, either in the levels of GSH or of lipid peroxidation in the brain of the rat. A possible explanation for this difference with our results could be the fact that the authors studied only microsomal lipid peroxidation of the whole brain and, according to Noda,[26] regional differences can mask the day–night changes reported here.

We can try to discuss the physiological meaning of our results in the light of other authors' findings. Lipoperoxidation modifies the unsaturation of membrane lipids and modulates the activity of several enzymes, such as SOD or adenylate cyclase as has been reported respectively by Badwey[3] and Baba.[1,2] Lipoperoxidation may also affect prostaglandin production or the turnover of the fatty acids of the membrane phospholipids, since Hemler *et al.*[18] showed that lipoperoxidation of polyunsaturated fatty acids might regulate prostaglandin biosynthesis and Mead[25] has recently reported that epoxy derivates of the fatty acids from membrane phospholipids facilitate phospholipase action. The phenomena described are related to light and darkness and perhaps to some physiological functions synchronized by the photoperiod, such as fasting and feeding or the rest and activity periods of the rat. The possible cause–effect relationship has to be tested by further experiments.

We propose that the observed fluctuations in lipoperoxidative activity in the cerebral cortex of the rat are an oscillatory phenomenon in the nervous system, and could play a role in modulating the membrane properties of the nervous cell. Further studies are needed to understand the nature of these fluctuations.

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