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RAPID COMMUNICATION

Circadian Oscillations of Lipid Peroxides in the Rat Pineal Gland

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Summary

We studied the circadian oscillation of lipid peroxides (TBARS) in the pineal gland of rats adapted to light:dark 12:12 h regimen. The concentration of TBARS was determined at 3-h intervals during 24 hours. TBARS of pineal gland oscillated rhytmically during the 24 h period. The maximal concentration of lipoperoxidative products was found at 20.00 h and 02.00 h and the lowest values at 08.00 h and 23.00 h. The determination of antioxidant capacity is needed for explaining the mechanism of TBARS oscillations in the pineal gland.

Key words

Rat – Pineal – Lipid peroxides – Oscillations

Under physiological conditions, reactive oxygen compounds and the associated free radicals are routinely produced by cells such as eosinophils, macrophages, monocytes and neutrophils, mainly in relation to the elimination of bacteria and other microorganisms. Under certain conditions, low levels of free radicals function as intracellular secondary messenger (Schreck and Bauerle 1991, Schreck *et al.* 1991) to maintain homeostasis in the organism. Besides that, free oxygen radicals initiate the peroxidation of lipids, cause damage to cellular membranes and other cell organelles including receptors that they may contain. Polyunsaturated fatty acids (PFA) present in membranes are subject to hydroxylation which can occur not only by peroxidation but also from the lipoxygenase metabolic pathway (Kim *et al.* 1991). The activity of cyclooxygenase (prostaglandin synthetase) and 15-lipoxygenase (Cardinali and Pita 1983) was demonstrated in the pineal gland. Arachidonic and docosahexaenoic fatty acids, the presence of which have been undoubtedly proved in the pineal gland (Sarda *et al.* 1991), are exposed to the action of free oxygen radicals similar to that exerted on the same fatty acids in other tissues. Besides that, the release of hydroxyl radicals *via* the lipoxygenase and cyclooxygenase pathways of arachidonic acid conversion was observed (Bergendi 1988).

On the basis of our previous experiments (Solár *et al.* 1995) and the knowledge about the presence of arachidonic acid and its metabolites in the pineal gland, we decided to determine the quantity of lipide peroxides in the form of products of thiobarbituric acid reactive substances (TBARS) and their circadian oscillations in the pineal gland homogenates.

Male Wistar SPF rats (Velaz, Prague) weighing 180–200 g with free access to food and water were used. Rats were adapted to a light regimen (LD 12:12, light with an intensity of 150 lux in the cage from 07.00 to 19.00 h) for 4 weeks. Eight groups of animals, each consisting of 7–8 rats, were analyzed within 24 hours at 3-h intervals. The animals were killed at 08.00 h, 11.00 h, 14.00 h, 17.00 h, 20.00 h, 23.00 h, 02.00 h and 05.00 h. The concentration of lipid peroxides in pineal gland homogenates was determined according to Satch (1978). The absorbance of TBARS was measured spectrophotometrically at the wavelength of 535 nm. Values of TBARS are reported as malondialdehyde equivalents and its formation was quantified using a molar extinction coefficient of 1.49×10^5 $\mu mol^{-1}.cm^{-1}$. The results were evaluated using the Peritz's multiple

F-test (Harper 1984) and population – mean cosinor analyzis (Halberg *et al.* 1967).

TBARS of pineal gland oscillated rhythmically during the 24 h period. One hour after the onset of light, the lipid peroxidation began to increase until 02.00 h, with the exception of 23.00 h, when the TBARS value was decreased. The maximal concentration of lipoperoxidative products were found at 20.00 h and 02.00 h. TBARS began to decrease after 02.00 h and reached its lowest value at 08.00 h. The acrophase was localized at 3 h and 25 min after the beginning of the dark part of the day, i.e. at 22.25 h (Fig. 1). The characteristics of the cosinor test (the values of mesor, amplitude and acrophase) are given in Table 1.

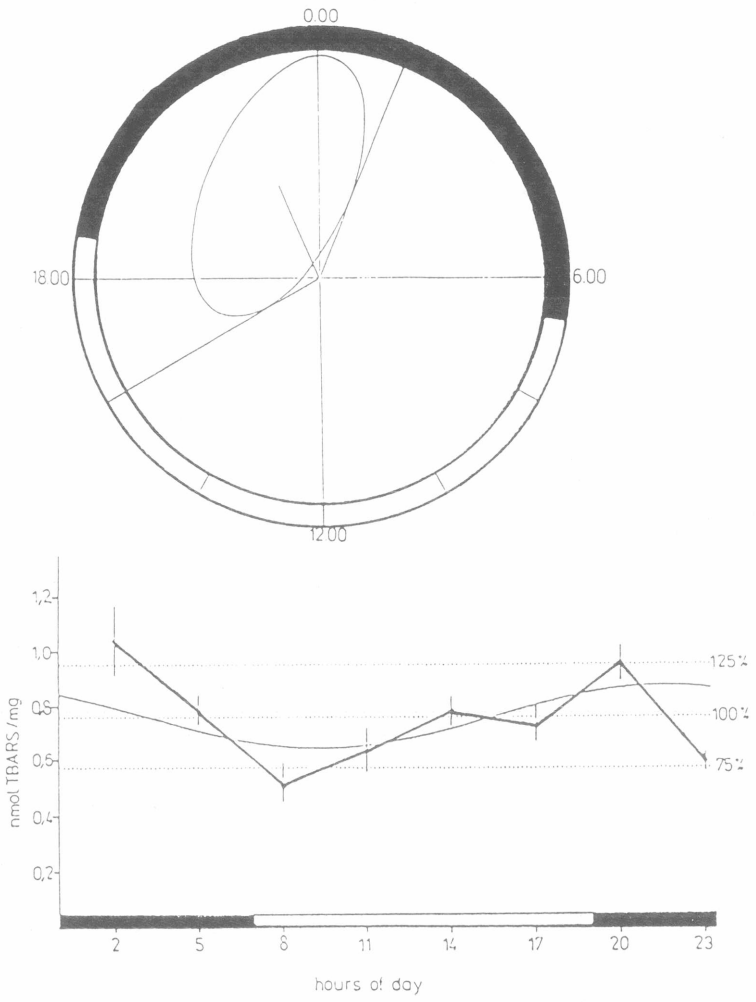

Fig. 1. *Circadian oscillations (means ± S.E.M.) and cosinor diagram (circular plot) of lipid peroxides in the pineal gland. Empty part of the band – light and full part – darkness. The basal characteristics of oscillations are illustrated on the cosinor diagram. The vector originating from the centre of the circular system of coordinates represents the amplitude of oscillations. The orientation of the vector indicates the acrophase on the circular scale (in hours). The ellipse drawn with centre at the end of the vector represents the confidence area ($P<0.05$) for the acrophase and amplitude. The tangents to the ellipse delimitate the confidence interval for the acrophase ($P<0.05$). If the ellipse does not overlap the origin, the rhythm with a chosen period is present. The thick line, in the curve, represents the experimental values given as means ± S.E.M. and the thin line represents approximated model function. The values are expressed as MDA concentrations in nmol/mg (on the left) and as % of the overall mean (on the right).*

Table 1. Characteristics of the cosinor test

Tissue	Period of the rhythm (hours)	Mesor ± S.E.M. (nmol MDA/mg)	Amplitude ± CI (95 %) (nmol MDA/mg)	Acrophase ± CI (95 %) (hours)
Pineal gland	24	0.754±23.6	0.114 (0.017;0.211)	22.25 (16.07;01.30)

CI – confidence interval; its limits are given in bracket

In comparison with other tissues, the brain contains high quantities of PFA, mostly arachidonic and docosaexaenoic fatty acids (Koudelová and Mourek 1994). Sarda *et al.* (1991) stated that arachidonic and docosahexaenoic acid belong to the PFA that most frequently occur in the pineal gland.

The high content of PFA offers a large number of double bonds which are easily attacked by free oxygen radicals. This results in peroxidation of polyunsaturated fatty acids and development of degradation products. Besides other methods, the frequently used determination of TBARS (Esterbauer *et al.* 1991, Janero 1990) serves as an index of lipid peroxidation. In their experiments dealing with circadian oscillations of lipid peroxides in the rat cerebral cortex, the highest cortical lipoperoxidase activity were observed in the night hours (20.00–04.00). In our experiment, the highest concentration of TBARS were similarly recorded in the pineal gland between hours 20.00 and 02.00. The increase in cortical lipoperoxide products was associated with a decrease in glutathione peroxidase activity and an increase in superoxide dismutase activity (Diaz-Muñoz *et al.* 1985). No literary data are available on the activity of antioxidative enzymes in the pineal gland. Although the presence of arachidonic and docosahexaenoic fatty acids as well as of their hydroxyacids in the pineal gland (Sarda *et al.* 1991, Sawazaky *et al.* 1994) is considered to be meaningful, it is necessary to supplement the available data with the information about circadian oscillations. Only such information will allow us to determine the presence or absence of correlation between TBARS and oscillations in the concentration of arachidonic and docosahexaenoic fatty acids (or their hydroxyacids) in the pineal gland.

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