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Lipid Peroxidation in Isolated Membranes of Cerebral Cortex, Heart and Kidney

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Summary

The extent of ADP.Fe/NADPH-induced lipid peroxidation measured as production of thiobarbituric acid-reactive substances (TBARS) was determined in isolated membranes from cerebral cortex, heart and kidney of 21-days-old rats. The time course of lipid peroxidation showed higher production of TBARS in cerebral cortex than in heart and kidney. Our data indicate that high level of TBARS production is not due to high activity of NADPH oxidoreductase but due to high content of endogenous lipids in cerebral cortex membranes that could be modified. Higher production of TBARS in cerebral cortex is the result of higher content of lipids in cerebral cortex membranes because NADPH cytochrome c reductase activity in membranes of cerebral cortex is lower than that of heart and kidney.

Key words

ADP.Fe/NADPH-induced lipid peroxidation - Cerebral cortex - Heart - Kidney - NADPH cytochrome c reductase

Introduction

Oxygen is essential substance in cell energy metabolism but oxygen radicals formed by interaction of oxygen with free electrons are toxic substances. They cause the destruction of biological membranes and impair the normal cell function. An abnormal membrane function may be an early indicator of free radical-induced injury in living cells. Therefore the generation of oxygen radicals followed by lipid peroxidation has become a focus of attention for many investigators. Lipid peroxidation is a complex process in which polyunsaturated fatty acids in biological membranes undergo degradation by a chain of reactions and form lipid hydroperoxides which decomposed double bounds and destruct membrane lipids to yield epoxy-fatty acids, alkanes, alkenes, alkanals, alkenals, 4-hydroxyalkenals and aldehydes (including malondialdehyde) (Sunderman 1986). The changes of lipid peroxidation ultimately lead to modifications of the structural and functional integrity of biological membranes and of the whole cell systems (Frank and Massaro 1980). The high resistance of newborn mammals to oxygen deficiency is well known for a long time. However, the resistance of central nervous system to oxygen deficiency diminishes from 2nd to 21st day and rats aged 21 days show the lowest resistence to a lack of oxygen as anoxia, hypoxia and ischaemia (Mourek 1958, Koudelová and Mourek 1992). During the ontogeny of rat the age of 21 days can be characterized as a quantitative growth. From the aspect of nutrition, the onset of weaning (which is determined as a phase of life when the young animal gradually passes from maternal milk to solid food) starts on about the 15th day of life and terminates at the end of the fourth postnatal week when the young stop taking maternal milk so that the 21st day is just in the middle of the weaning period (Babický *et al.* 1973a,b).

As regards the sensitivity to oxygen radicals, nervous system is particularly susceptible to oxygen radical damage because of the high levels of polyunsaturated fatty acids in phospholipids that are comprised in neuronal cell membranes which serve the uniquely specialized functions (Braughler and Hall 1989).

In our previous paper we have found content of endogenous lipoperoxides in different parts of brain from 21-day-old rat (Koudelová and Mourek 1991). In this present study we have evaluated the maximum capacity for lipoperoxide formation and we have also compared to which extent the capacity of cell membranes for *in vitro* induced lipoperoxide formation varies in different tissues.

Methods

All used chemicals were of the purest grades available commercially. NADPH, ADP, thiobarbituric acid, Tris(hydroxymethyl)aminomethane (Tris) and cytochrome c (from bovine heart) were obtained from Sigma (USA), other chemicals from Lachema (Czech Republic).

The experimental animals were Wistar strain rats of both sexes of the different age of (7 days, 21 days and 3 months old) from breeding colony of Faculty of Medicine. Rats were killed by decapitation. Heart, kidney and brain were quickly removed into ice-cold 250 mmol/l sucrose-10 mmol/l Tris-HCl, pH 7.4. Cerebral cortex was separated on the cold block.

The total membranes of cerebral cortex, heart and kidney were isolated from 10 % frozen-thawed homogenates by centrifugation for 60 min at 100 000 x g in a Beckman L50 centrifuge. Pellets were suspended in 250 mmol/l sucrose-10 mmol/l Tris-HCl, pH 7.4.

The assay system for enzymatic, ADP.Fe/NADPH-induced lipid peroxidation contained 150 mmol/l KCl with 20 mmol/l Tris-HCl buffer, pH 7.4, 225 μmol/l NADPH, 3 mmol/l ADP, 50 μmol/l $FeCl_3$ and 0.5 mg membrane protein. The assay system for non-enzymatic, ascorbate-induced lipid peroxidation contained 50 μmol/l $FeCl_3$ and 0.4 mol/l ascorbic acid and 0.5 mg membrane protein in the above mentioned buffer. After incubation at 37 °C in a shaking water bath, the thiobarbituric acid-reactive substances (TBARS) were estimated by using thiobarbituric acid method. One ml of incubation suspension was mixed with 1 ml of a solution containing TCA-TBA-HCl reagent (Buege and Aust 1978). The mixture was allowed to proceed in the dark for approximately 20 h at room temperature as described Erdahl *et al.* (1991). The samples were centrifugated to remove precipitate and the absorbance was determined at 535 nm. Values for TBARS are reported as malondialdehyde (MDA) equivalents similarly as was done by Buege and Aust (1978) for microsomal lipid peroxidation experiments. MDA formation was quantitated using a molar extinction coeficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nanomoles MDA per mg membrane protein.

The activity of NADPH cytochrome c reductase (E.C. 1.6.2.4.) was measured according to Sottocasa *et al.* (1967).

The activity of cytochromoxidase (E.C. 1.9.3.1.) was measured as described before (Kalous *et al.* 1989).

The lipid phosphorus was determined from chloroform-methanol extracts according to Bartlett (1959). Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Results

Table 1 shows that there were no age-dependent differences in ADP.Fe/NADPH-induced TBARS production by isolated cerebral cortex membranes incubated for 30 min. Values of 7-day-old and adult rats were similar to those obtained for 21-day-old animals. We have also found no differences between males and females.

Table 1
Level of TBARS in isolated membranes from cerebral cortex of rats of different rat age

Sex	Age of rat 7 days	21 days	Adult
Male	23.00 ± 1.46 (4)	18.82 ± 3.31 (9)	24.21 ± 1.66 (3)
Female	22.70 ± 0.41 (4)	19.25 ± 3.11 (9)	19.10 ± 6.57 (3)

TBARS levels are expressed as nmoles MDA/mg membrane protein. The results are given as the means ± S.D. The number in brackets indicates number of experiments.

Table 2 documents the level of endogenous TBARS in isolated total membranes of cerebral cortex, kidney and heart and the level of TBARS after 30 min incubation with ADP.Fe/NADPH. We can see differences among membranes isolated from different organs but we have not obtained significant differences between male and female rats. The values obtained for kidney and heart membranes have been always lower than those for cerebral cortex.

The rate of TBARS production during *in vitro* induced peroxidation is not linear as demonstrated in rat liver microsomes (Devasagayam 1986), rat liver mitochondria and mitoplasts (Szabados *et al.* 1987). Therefore we have monitored the time course of TBARS production of ADP.Fe/NADPH-induced lipid peroxidation in isolated membranes from cerebral cortex, kidney and heart (Fig. 1).

Table 2
Level of TBARS in isolated membranes from cerebral cortex, kidney and heart of 21-day-old rats

Tissue	Sex	Level of Endogenous TBARS (A)	Level of ADP.Fe/NADPH induced TBARS (B)	Induced peroxidation (B−A)
Cerebral cortex	M	0.84±0.34 (9)	18.82±3.31 (9)	17.98
	F	0.77±0.29 (9)	19.25±3.11 (9)	18.48
Kidney	M	0.25±0.15 (5)	6.93±2.57 (5)	6.68
	F	0.16±0.03 (5)	6.96±3.13 (5)	6.80
Heart	M	0.50±0.20 (4)	10.21±2.84 (4)	9.71
	F	0.58±0.13 (4)	10.47±1.92 (4)	9.89

TBARS levels are expressed as nmoles MDA/mg membrane protein. They indicate the level of endogenous content of TBARS (A) and the level of ADP.Fe/NADPH induced TBARS produced during 30 min incubation at 37 °C. The results are given as the means±S.D. The number in brackets indicates number of experiments. M means male, F means female

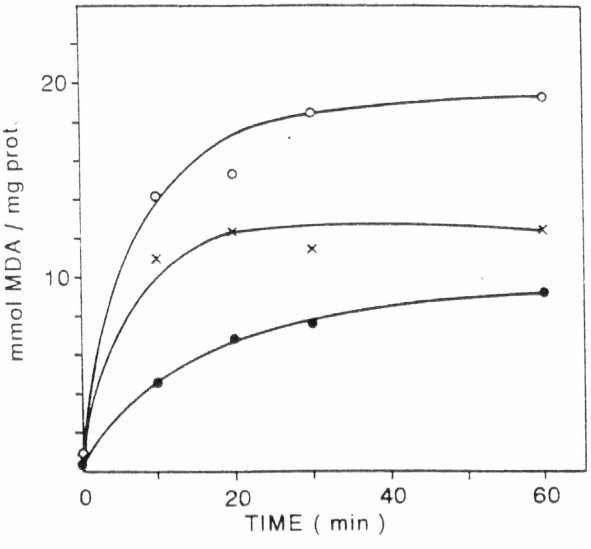

Fig. 1
Time course of ADP.Fe/NADPH-induced lipid peroxidation in cerebral cortex, heart and kidney membranes. Incubations were carried out as described in section Material and methods using full complement of cofactors for ADP.Fe/NADPH-induced peroxidation.

As we can see most of the TBARS production occurs within 30 min of incubation in all three tissues. Further increase during 30-60 min is very limited in all organs tested. For non-enzymatic ADP.Fe/ascorbate-induced TBARS production we have obtained higher values than for enzymatic one, however the differences among various organs were the same (data not shown).

Table 3
The activity of NADPH cytochrome c reductase and cytochrome c oxidase and lipid phosphorus content in isolated membranes from cerebral cortex, kidney and heart of 21-day-old rats

Tissue	NADPH Cytochrome c Reductase	Cytochrome c Oxidase	Lipid Phosphorus Content
Cerebral cortex	8.02±0.45	0.297±0.03	6.8±1.3
Kidney	12.49±1.02	0.505±0.09	3.5±0.58
Heart	6.26±0.73	0.454±0.05	3.6±0.68

NADPH cytochrome c reductase activity is expressed as nmoles cytochrome c/min/mg membrane protein, cytochrome c oxidase is expressed as μmole cytochrome c/min/mg membrane protein, lipid phosphorus content is expressed as μg/mg membrane protein. The results are given as the means ± S.D. The number of measurements in the individual groups was at least 6.

In our experimental conditions of enzyme-induced lipoperoxide formation NADPH oxidoreductase is one of the most important factors in TBARS generation. The data in Table 3, however, demonstrate that the highest activity of this enzyme is in the kidney where we have found the lowest lipoperoxides formation (Tables 2 and 3).

In intact cells mitochondria are considered as a generator of free electrons which can be involved in lipoperoxide formation (Turrens and Boveris 1980, Glinn *et al.* 1991). The level of cytochrome c oxidase activity, marker of mitochondrial inner membrane, however, showed that cerebral cortex containes lower activity then kidney and heart (Tab. 3).

We have also analysed the content of lipid phosphorus in cell membranes (Table 3). As we have expected, the highest lipid phosphorus content is in cerebral cortex membrane preparations.

Discussion

In our study, we have used the production of thiobarbituric acid reactive substances for measuring of lipid peroxidation in biological samples despite the considerable criticism of its limitations. Nevertheless, the level of TBARS production remains a reliable index of lipid peroxidation and this method is the method of choice and the most frequently used method in this field (Janero 1990, Esterbauer *et al.* 1991).

Generation of free radicals and lipoperoxide formation are now accepted to disturb different membrane functions and to be related to a variety of pathological states, cancer and aging. In this process a few types of events are involved: the rate of free electrons release and oxygen radicals generation, the efficiency of enzymatic and non-enzymatic antioxidants protection system and the quantity of endogenous substrates that can be modified by free radicals (e.g. unsaturated free fatty acids).

Lipid peroxidation studies *in vitro* are useful for the elucidation of possible mechanisms of peroxide formation *in vivo*. The aim of our study was to evaluate the capacity of biological membranes of cerebral cortex, heart and kidney to react with oxygen radicals and follow lipoperoxides formation in membrane preparations where all cytosolic antioxidative defence systems were eliminated.

We may confirm that the highest level of TBARS correlates well with the content of lipid phosphorus estimated in cerebral cortex membranes in comparison with membranes from heart and kidney. This suggests that cerebral cortex cells should be better equipped by endogenous antioxidant defense system to prevent their membranes against peroxidative damage. We may conclude from our experiments that inducers of enzymatic and non-enzymatic lipid peroxidation produce in cerebral cortex membranes *in vitro* the highest quantity of TBARS from all tested tissues. Our results thus present further evidence to previous data indicating that brain structures are very sensitive to oxygen radicals (Braughler and Hall 1989).

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Reprint Requests

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