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The influence of oxidative stress on the level of malondialdehyde (MDA) in different areas of the rabbit brain

Several lines of evidence indicate that lipid oxidation by free radicals and accumulation of oxidatively modified lipids, have been found in cells, during ageing and oxidative stress. Lipid peroxidation has been implicated in various pathological conditions, including rheumatic diseases, termal injuries, atherosclerosis, muscular dystrophy and drugs toxicity. The brain is an organ which has both high oxygen consumption and high concentration of unsaturated lipids and catecholamines and other compounds, which can be readily oxidised. Autooxidation of the brain is probably the consequence of its high content of lipids and high superoxide radicals production.

In this study we measured lipid peroxidation in three brain areas: brain hemisphere, brainstem and cerebellum. We measured levels of malondialdehyde (MDA), which is one of the lipoperoxidation products of polyunsaturated fatty acid before and after oxidative stress.

MATERIAL AND METHODS

Eight-week New Zealand rabbits were used as experimental material. After decapitating the animals, the brain was isolated and the brain hemispheres, brainstem and cerebellum were prepared. The homogenization of tissues was carried out in 0.9% NaCl at $+4^0$C. The 10% homogenate was later centrifuged for 20 minutes (3,500 rev/min). The 10% homogenate and supernatant were used to determine the level of malondialdehyde (MDA) according to the Ohkawa method (12). Protein was determined according to the Lowry method with some modification (10). The reaction mixture contained 0.2 ml – 0.8 ml of sample, 0.2 ml of 8.1% sodiumdodecyl sulphate (SDS), 1.5 ml of 20% acetic acid solution of 3.5 pH, 1.5 ml of 0.8% aqueous solution of TBA. The mixture was finally completed to 4.0 ml with distilled water and heated at 95^0C for 60 min. After cooling

with tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1, v/v) were added, and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the absorbency of the organic layer (upper layer) was measured at 532 nm.

TMP (1.2.3.3 – tetramethoxypropane) was used as an external standard and the level of lipid peroxides was expressed as nmol of MDA. Lipid peroxidation was studied *in vivo* by subjecting homogenates and supernatants of brain hemispheres, brainstem and cerebellum to oxidative stress using free radical generating system. A simple peroxidation mixture consists of tissue homogenate: 50 μl 0.1 mM NADPH, 50 μl 0.05 mM $FeCl_3$, 100 μl 0.8 mM ADP. After incubation at 25^{0}C for 20 min the lipid peroxidation was interrupted by adding 8.1% SDS. Lipid peroxidation was followed by measuring the free MDA as described above. The results are expressed as nanomoles of MDA formed in this reaction per milligram of protein (10). Statistical difference was tested by paired Student's t-test. $P<0.05$ values were considered statistically significant.

RESULTS AND DISCUSSION

Free radicals are formed in the central nervous system (CNS) as part of normal metabolic processes (3). High oxygen uptake and low antioxidant defences increase the vulnerability of the CNS to oxidative damage. Namely, it is known that during lipids peroxidation reaction, hydroxyl radicals can react with polyunsaturated fatty acids incorporated in cellular membranes, leading to the formation of peroxy radicals on the intermediates, thus perpetuating free radicals reactions (5). The final result is structural damage to membranes and degeneration. As free radicals are difficult to estimate directly *in vivo*, because of their high reactivity and short half-life, the level of a stable product of lipid peroxidation malondialdehyde (MDA) was used as a measure of oxidative stress in homogenates and supernatants of brain hemispheres, brainstem cerebellum of rabbit brain.

Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of peroxidation reaction. Malondialdehyde has been identified as the product of lipids peroxidation (7). Our results showed differences in levels of peroxidation products in supernatant and homogenate in different parts of rabbit brain (Table 1). Lipid structure contents in supernatant was significantly more sensitive for oxidative stress than lipids of homogenate. Components of membranes, phospholipids, polyunsaturated fatty acids are converted by peroxidation to MDA. This has changed stability and function for membranes. These findings showed an important role of oxidative stress.

In conditions of oxidative stress, levels of MDA were increased significantly in comparison to levels of control. In supernatant the level of MDA in brain hemispheres was increased twice, in brainstem three times in comparison to control (Table 2). In homoge-

Table 1. Comparison of the concentrations of MDA (nmol/mg protein) in supernatant and homogenate, in different areas of rabbit brain. All data are the mean ± SD (N=10)

	Supernatant	Homogenate	Test t
Brain hemisphere	1.52±0.22	5.21±0.84	p<0.01
Brainstem	1.19±0.23	4.44±0.14	p<0.01
Cerebellum	1.23±0.13	5.51±0.34	p<0.01

Table 2. Concentration of MDA (nmol/mg protein) in supernatant of different areas of rabbit brain. All data are the mean ± SD (N=10)

	Control (after 20-minute incubation)	After 20-minute incubation with oxidative stress	Test t
Brain hemisphere	2.47±0.23	6.60±1.33	p<0.01
Brainstem	2.37±0.11	7.87±0.62	p<0.01
Cerebellum	2.86±0.17	7.81±0.87	p<0.01

nate the concentration of MDA was significantly increased only in brain hemispheres three times in comparison to control (Table 3). All these results clearly indicate differences susceptibility of different parts of rabbit brain for oxidative stress. The brain may be particularly vulnerable to oxidative stress. There are several means by which excess free radicals may be generated in the brain (11). The brain hemisphere may be particularly at risk for radical-induced damage because it contains large amounts of iron (which can be associated with increased free radical production through the Fenton reaction). Iron may serve as a catalyst for the formation of $^{\cdot}OH$ radicals from H_2O_2 (4). The potential role of free radicals metabolism in oxidative stress, seems to be known in a lot of

Table 3. Concentration of MDA (nmol/mg protein) in homogenate of different areas of rabbit brain. All data are the mean ± SD (N=10)

	Control (after 20-minute incubation)	After 20-minute incubation with oxidative stress	Test t
Brain hemisphere	7.37±0.25	22.08±5.90	p<0.01
Brainstem	5.69±0.27	11.98±1.61	p<0.01
Cerebellum	5.75±0.30	12.97±0.84	p<0.01

neurodegenerative diseases – multiple sclerosis, Parkinson's disease, Down syndrome (2, 6, 8). The human body has a complex antioxidant defense system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH Px), and catalase (CAT) (9, 13). The non-enzymatic antioxidant consists of molecules such as glutathione, α-tocopherol, ascorbic acid, and β-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions. Free radicals can stimulate chain reactions by interacting with proteins, lipids and nucleic acids causing cellular dysfunction and even death (1).

In conclusion, our findings seem to support a key role of oxidative stress reactions in structural damage, cell degeneration in different parts of the rabbit brain.

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SUMMARY

Lipid peroxidation is a process which is determined by the extent of peroxide-forming free radicals mechanism and the peroxide-removing antioxidant system. To assess the peroxidation process, products of lipid peroxidation are analysed. Products of lipid peroxidation, such as thiobarbituric acid (TBA) are defined as thiobarbituric acid reactive material. In the present study we measured lipid peroxidation in three areas of the rabbit brain: brain hemispheres, brainstem and cerebellum. Supernatant and 10% homogenate were used to determine the level of malondialdehyde (MDA) according to the Ohkawa method. We measured levels of MDA before and after oxidative stress. Our results showed an important role of oxidative stress, because levels of MDA were increased significantly in comparison to the level of control, in all different areas of the rabbit brain.

Wpływ stresu oksydacyjnego na poziom dialdehydu malonowego (MDA) w różnych częściach mózgu królika

Wolne rodniki tlenowe powodują autokatalityczną peroksydację lipidów zawierających wielonienasycone kwasy tłuszczowe. Prowadzi to do powstania licznych organicznych wolnych rodników, a także dialdehydu malonowego (MDA). Mózg zużywa codziennie około 20% tlenu i jest bogatym źródłem wolnych rodników. W obecnej pracy podjęto próbę oceny wrażliwości mózgu królika na indukowany szok tlenowy. Badaniem objęto trzy obszary mózgu: półkule mózgowe, pień mózgu i móżdżek. Wpływ szoku tlenowego na peroksydację lipidów w powyższych obszarach określano metodą Ohkawy poprzez pomiar stężenia MDA w nmol/mg białka. Otrzymane wyniki pozwalają zaobserwować znaczne podwyższenie MDA we wszystkich obszarach mózgu królika pod wpływem stresu oksydacyjnego (szczególnie w pniu mózgu we frakcji supernatantu i w homogenacie kory mózgowej).