MEMBRANES OF SUBCELLULAR ORGANELLES AS A SOURCE OF SUPEROXIDE RADICALS IN ISCHEMIA OF THE LIVER

L. S. Vartanyan, Yu. É. Rashba, L. G. Nagler, A. A. Zgurskii, L. A. Oktyabr'skaya, and M. V. Bilenko

UDC 616.36-005.4-092:616.36-008.922.1]-07-092.9

KEY WORDS: ischemia of the liver; superoxide radicals; mitochondria; microsomes; nuclei

One of the most actively studied forms of pathology, for which a significant role of oxygen radicals has been established in its pathogenesis, is ischemia of various organs followed by reperfusion [14, 15]. However, the problem of the main sources of origin of the oxygen radicals and of the free-radical mechanisms of development of damage induced by ischemia and reperfusion, remains a matter for debate.

In the investigation described below quantitative data were obtained on the effect of total ischemia in vivo on the rate of formation of superoxide radicals ($O_2^{\cdot-}$) in microsomal, mitochondrial, and nuclear membranes of the liver, and the rates of formation of $O_2^{\cdot-}$ radicals (V) were compared with activity of the corresponding superoxide dismutase (SOD), with allowance for its compartmentalization.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-220 g. Total ischemia of the liver was induced by applying microforceps to the vascular pedicle of the central and left lateral lobes of the liver for 2 h. Reperfusion was carried out for 24 h after 2 h of ischemia and the nonischemic lobes (about 30%) were resected immediately after the end of ischemia. Not only the liver of intact rats, but also the liver of rats after hepatectomy with resection of a similar weight of the organ, served as the control. The subcellular fractions were isolated as described previously [3, 7, 8]. SOD activity in the cytosol was determined as in [2]; in the mitochondria as in [10, 12]. The rate of $O_2^{\cdot-}$ generation was measured as oxidation of 2,2,6,6-tetramethyl-4-oxo-piperidine hydroxylamine (HA) to the corresponding stable nitroxyl radical, recorded by the EPR method [3, 7, 8]. Measurements of V for all membranes were made in 0.1 M phosphate buffer, pH 7.4, containing $5 \cdot 10^{-4}$ M diethylenetriaminepenta-acetic acid, and in microsomes and nuclei — with a regenerating system containing 10^{-4} M NADPH and $3 \cdot 10^{-4}$ M glucose-6-phosphate dehydrogenase, and in submitochondrial particles (SMP) — with $4 \cdot 10^{-4}$ M NADH, $8 \cdot 10^{-3}$ M succinate, and, as inhibitors, $1.5 \cdot 10^{-6}$ M rotenone and $9 \cdot 10^{-6}$ M antimycin A. The HA concentration in the experiments with microsomes was $2 \cdot 10^{-3}$ M, in those with SMP $5 \cdot 10^{-3}$ M, and in those with nuclei $1.6 \cdot 10^{-2}$ M. The rate of oxygen uptake was measured under the same conditions on a "YSI Model 53" polarograph (USA) with a Clark's electrode.

EXPERIMENTAL RESULTS

One of the principal sources of superoxide radicals in the body is the electron transport chain in the various subcellular organelles. However, no comparable quantitative data on the rates of $O_2^{\cdot-}$ formation in different membranes can yet be found in the literature. The use of a new method, applicable to all types of membranes, has enabled such data to be obtained.

Institute of Chemical Physics, Academy of Sciences of the USSR. Drug Research Institute, Ministry of the Medical and Biological Industry, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. A. Vladimirov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 550-552, June, 1990. Original article submitted July 7, 1989.

TABLE 1. Oxygen Uptake and O_2^- Formation in Various Subcellular Organelles of Intact Rat Liver ($M \pm m$)

Organelle	Oxygen uptake, nmoles/min/mg protein	V, nmoles/min/mg protein
Microsomes	2,67±0,14	2,0±0,14
SMP	10,0±0,49	0,20±0,02
Nuclei	—	0,50±0,09

TABLE 2. Effect of Ischemia and Reperfusion of Rat Liver on V (in relative units compared with control, taken as 1) and a (in percent) in Various Subcellular Organelles ($M \pm m$)

Experimental conditions	Microsomes		SMP		Nuclei	
	V	α	V	α	V	α
Control (intact rates)	1,0	75,2±0,9	1,0	2,0±0,11	1,0	2,0±0,25
2 h of ischemia	0,64±0,03**	75,0±0,03	1,45±0,13**	3,35±0,16*	1,13±0,26	2,0±0,30
2 h of ischemia +24 h of reoxygena	0,41±0,10**	74,3±1,9	1,66±0,21**	3,5±0,22*	0,81±0,25	2,0±0,36
24 h after partial hepatectomy	1,0±0,12	—	0,56±0,15	—	0,70±0,14	—

Legend. $*p < 0.05$, $**p < 0.01$ compared with control.

It was found that O_2^- radicals are formed most quickly in microsomal membranes, but that the rate of generation of O_2^- in the nuclear and mitochondrial membranes is 5-10 times slower (Table 1). According to these data the ratio of V to the rate of oxygen uptake (α, %), which characterizes the contribution of processes of single-electron reduction of oxygen, was about 2% for SMP, whereas previously a value of about 3% was obtained for mouse liver nuclei [3]. In the present experiments with microsomes, in the absence of exogenous substrates oxygen uptake and O_2^- radical formation were determined by reactions of free oxidation of NADPH. Since under the conditions studied the value of α in the microsomes was about 75%, this means that mainly single-electron transfer takes place in the hydroxylation chain. During free oxidation of NADPH, O_2^- formation in the microsomes can eyidently take place on account of autooxidation of the flavoprotein and of cytochrome P-450 [1, 13].

Data on the effect of ischemia and reperfusion on the rate of O_2^- formation are given in Table 2. During ischemia and subsequent reperfusion, a steady fall in the value of V took place in the microsomes, in harmony with the fall in the cytochrome P-450 concentration [6]. In the nuclei V did not change significantly during either ischemia or reoxygenation, allowing for the fact that, because of the nature of the model of ischemia used, the value of V in the nuclei may be reduced during reperfusion because of the effect of partial hepatectomy.

In SMP and during both ischemia and reperfusion, V increased by about 1.5 times compared with the control. If it is recalled that partial hepatectomy leads to a significant fall in the values of V in SMP, the true value of V in the reperfusion period must evidently be higher than that observed. Incidentally, in the experimental model used, all operations following ischemia in vivo were carried out in air. Under these circumstances the results only of irreversible disturbances in the electron transport chains are recorded. Whereas changes in SMP during ischemia were reversible in character, as a result of reoxidation of the liver in vitro no changes could be found in the value of V during isolation of SMP. Increased O_2^- formation during cardiac ischemia in vitro also was observed in [5], but on whole mitochondria, without separation of the Mn-SOD mitochondrial matrix.

In the case of SMP, as a result of ischemia and subsequent reperfusion, the value of α also increased. For microsomes and nuclei, α did not differ from the corresponding value for organelles isolated from the liver of control animals.

During ischemia a decrease was found previously in Cu,Zn-SOD activity [4]. In agreement with these data we also observed a significant lowering of Cu,Zn-SOD activity in the cytosol during 2 h of ischemia, and some recovery took place in the 24 h of reperfusion which followed (Table 3). Partial hepatectomy did not affect Cu,Zn-SOD activity. Activity of Mn-SOD was virtually unchanged during ischemia, but it must be particularly emphasized that reperfusion almost doubled Mn-SOD activity. If it is recalled that partial hepatectomy leads to the opposite effect (activity of Mn-SOD fell), a purely reperfusion effect evidently causes an even greater increase in Mn-SOD activity.

TABLE 3. Effect of Ischemia and Reoxygenation on Cu,Zn- and Mn-SOD Activity (in relative units, compared with control, $M \pm m$)

Experimental conditions	Cu, Zn-SOD activity	Mn-SOD activity
Control (intact rats)	1,0	1,0
2 h of ischemia	0,34±0,05	1,02±0,19
2 h of ischemia + 24 h of reper	0,57±0,07	1,80±0,07
24 h of partial hepatectomy	1.1±0,11	0,60±0,25

TABLE 4. Effect of Ischemia and Reperfusion of Rat Liver on Ratio between Rate of Formation of Superoxide Radicals and Superoxide Dismutase Activity in Various Subcellular Organelles

Experimental conditions	Microsomes	Submitochondrial particles	Nuclei
	V/Cu, Zn-SOD activity, relative units	V/total activity of mitochondrial (cu, Zn- and Mn-SOD), relative units	V/activity of Cu, Zn-SOD, relative units
Control (intact rats)	1,0	1,0	1,0
2 h of ischemia	1,88	1,88	3,3
2 h of ischemia + 24 h of reperfusion	0,72	0,97	1,42
24 h of partial hepatectomy	0,91	0,93	0,64

The SOD are known to be substrate-regulated enzymes [9]. As regards Mn-SOD it has also been shown that its activity in *E. coli* may be regulated by reduced glutathione, low concentrations of which induce Mn-SOD synthesis, whereas high concentrations inhibit it [11]. Measurements of glutathione and other reducing agents in stationary concentrations during reperfusion may evidently be the cause of Mn-SOD induction.

To judge the physiological role of changes found during ischemia and reperfusion, the rate of formation of O_2^- radicals was compared with Cu,Zn- and Mn-SOD activity (Table 4).

Although as the results in Tables 2 and 3 show, changes in Cu,Zn-SOD activity and V in the microsomes are similar in direction, the Cu,Zn-SOD activity of the cytosol fell substantially more than did V. This suggests that this uncompensated decrease in specific SOD activity in the cytosol may lead to an increase in the steady-state concentration of O_2^- radicals. In the mitochondria during ischemia the absolute value of V fell while activity of Mn-SOD remained virtually unchanged. Nuclei do not contain SOD of their own, and they utilize the Cu,Zn-SOD of the cytosol as a protective enzyme, During ischemia, although the value of V in the nuclei remained unchanged, because of the threefold decrease in cytosol SOD activity, the antioxidant protective system of the nuclei may be substantially impaired. Considering the closeness of the genetic material to the sources of O_2^- in these structures, the decrease in Cu,Zn-SOD activity during ischemia may represent a particular hazard for the nuclei.

During ischemia, therefore, an uncompensated relative increase in V takes place in all the subcellular membranes compared with the corresponding compartmentalized specific SOD activity. On reoxygenation the ratio between V and SOD activity returns close to normal. It can accordingly be concluded that O_2^- radicals formed in electron transport chains can make the greatest contribution to damage at the stage of ischemia itself and also, perhaps, in the early stages of reperfusion, i.e., by participating in the triggering mechanisms of ischemic damage. After prolonged reperfusion (24 h) this source of O_2^- evidently becomes unimportant.

LITERATURE CITED

1. A. I. Archakov, Microsomal Oxidation [in Russian], Moscow (1975).
2. L. S. Vartanyan and S. M. Gurevich, Vopr. Med. Khim., **88**, No. 5, 28 (1982).
3. L. S. Vartanyan and S. M. Gurevichr Biokhimiya, **54**, No. 5, 1020 (1989).
4. L. B. Dudnik, A. K. Tikhaze, et al., Byull. Éksp. Biol. Med., No. 5, 556 (1980).

5. A. M. Ledenev and E. K. Ruuge, Byull. Éksp. Biol. Med., No. 8, 303 (1985).
6. Yu. É. Rashba, L. S. Vartanyan, L. A. Seregina, et al., Byull. Éksp. Biol. Med., No. 11, 559 (1986).
7. Yu. É. Rashba, V. A. Chernikov, L. M. Baider, and L. S. Vartanyan, Biol. Membr., **3**, No. 8, 838 (1986).
8. Yu. É. Rashba, L. S. Vartanyan, L. M. Baider, and L. A. Krinitskaya, Biofizika, **34**, No. 1, 57 (1989).
9. I. V. Fridovich, Free Radicals in Biology [Russian translation], W. Pryor (ed.), Moscow (1979).
10. C. Beauchamp and I. Fridovich, Analyt. Biochem., **44**, No. 1, 276 (1971).
11. S. W. Bower and H. M. Hassan, J. Biol. Chem., **263**, 14,808 (1988).
12. H. Kuthan, H.-J. Haussman, and J. Werringloer, Biochem. J., **237**, 175 (1986).
13. R. I. Marray, M. T. Fisher, P. G. Debrunner, and S. G. Sliger, Metalloproteins, Part 1, Weinheim (1985), pp. 157-206.
14. J. M. McCord, Free Radicals Biol. Med., **4**, No. 1, 9 (1988).
15. M. L. Hess and N. H, Manson, J. Molec. Cell. Cardiol., **16**, No. 6, 969 (1984).

GANGLIOSIDE-DEPENDENT FACTOR INHIBITING LIPID PEROXIDATION IN SYNAPTOSOMAL MEMBRANES

Yu. Yu. Tyurina, V. A. Tyurin, N. F. Avrova, and V. E. Kagan

UDC 612.825.014:576.314].
015:577.125].064.085.2

KEY WORDS: liposomes; synaptosomes; gangliosides; lipid peroxidation

Experiments in vitro have shown that if standard systems are used to generate active forms of oxygen, significantly less accumulation of lipid peroxidation (LPO) products takes place in the plasma membranes than in the intracellular membranes (fraction of microsomes and mitochondria) [9]. Among the possible causes of resistance of the plasma membranes to LPO inducers at least three may be mentioned: the higher level of saturation of the phospholipids of these membranes and the fact that they contain predominantly glycolipids with a higher degree of saturation [2], the higher cholesterol concentration [3], and effective inhibition of LPO by an enzymic mechanism, coupled with activation of protein kinase C [10].

Gangliosides not only largely determine the structural and functional organization of the plasma membranes of cells, and play an essential role in processes of intercellular interaction and reception of biologically active substances, but they also take part in the regulation of differentiation, plasticity, and regeneration of nerve cells. However, the biochemical mechanisms of the neuronotrophic and neuroregenerative effect of gangliosides have not been elucidated [1].

It has recently been shown that gangliosides are modulators of the activity of various protein kinases, including protein kinase C [12]. With these considerations in mind, and also the fact that gangliosides are localized mainly in the outer monolayer of the plasma membranes of synaptosomes, where they may account for up to 10-15 moles % of the total lipids [4], the investigation described below was carried out to study the action of exogenous monosialoganglioside GM1 (accounting for about 30% of the total mammalian brain gangliosides) on synaptosomes of the rat brain.

EXPERIMENTAL METHOD

Synaptosomal membranes were obtained from the cerebral cortex of Wistar rats by the method described in [8], with certain modifications. The protein concentration was determined by a modified Lowry's method [11]. Gangliosides were extracted from hog brain by Folch's method, with additional treatment as described in [15]. Separation of the gangliosides into fractions

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. Institute of Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. A. Vladimirov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 553-555, June 1990. Original article submitted April 30, 1989.

