GANGLIOSIDES PROTECT ERYTHROCYTE MEMBRANES FROM MYOCARDIAL ISCHEMIA

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UDC 616.127-005.4-06.155.1]-07

KEY WORDS: erythrocytes; ischemia; myocardium; gangliosides; lipid peroxidation; Na^+,K^+-ATPase; lysophospholipids.

Acute circulatory disturbances in organs and tissues as a result of hypoxia were accompanied by activation of free-radical processes and of phospholipases A_2 [2, 3, 9]. Lipid peroxidation (LPO) products, as well as products of phospholipid hydrolysis by phospholipases A_2 (PLA_2), namely lysophospholipids and free fatty acids, are factors which modify the properties of the lipid bilayer and of some integral membrane proteins [2, 3, 12-14]. It was shown previously that besides antioxidants, gangliosides in vitro can also be involved in the regulation of LPO and can protect polyunsaturated fatty acids and enzymes from oxidative destruction in nerve tissue membranes [4, 15]. It has also been shown that gangliosides have a protective effect on membrane permeability during induction of LPO in an Fe-ascorbate system, and also in various forms of chemical damage [8]. A search for effective membrane protectors for use in stress-induced and ischemic damage, is currently in progress [2, 3].

The aim of this investigation was to study the action of gangliosides on the structural and functional organization of erythrocyte membranes in myocardial ischemia.

METHODS

Wistar rats weighing 200-250 g were anesthetized with phenobarbital sodium (70 mg/kg) intramuscularly. Experimental myocardial ischemia was created by ligation of the descending branch of the left coronary artery 2 mm below its base, for 15 min. Total bovine brain gangliosides (30 mg/kg) were injected intravenously 15 min before ligation of the artery. After decapitation of the animals, blood was collected in heparinized test tubes and erythrocytes were obtained also as described in [6] and then suspended in 50 mM Tris-HCl, 135 mM NaCl buffer, pH 7.4, or in bicarbonate buffer of the following composition (mM): NaCl 135, KCl 5, $MgCl_2$, $NaHCO_3$ 120, glucose 10, pH 8.4. To study the transmembrane distribution of amino phospholipids phosphatidylethanolamine (PEA) and phosphatidylserine (PS) the erythrocytes were incubated with 2.5 mM trinitrobenzenesulfonic acid (TNBS) at 0-4°C for 2 h. Lipids were extracted by Folch's method [10]. Isolation of modified amino phospholipids and fractionation of the phospholipids into classes were carried out by high-performance thin-layer chromatography, also as in [1]. Gangliosides were extracted and fractionated by preliminary column chromatography on silica-gel as described previously [4]. The end products of LPO of the Schiff's base type were recorded by their characteristic fluorescence (λ_{ex} = 365 mm, λ_{emis} = 420 nm) on an MFF-2A spectrofluorometer (Hitachi, Japan). Na^+,K^+-ATPase activity was determined as described in [6]. Erythrocytes were preincubated with monosialoganglioside GM_1 (10^{-8} M) in bicarbonate buffer at 37°C for 1.5 h. Erythrocyte membranes were treated with PLA_2 in bicarbonate buffer at 37°C

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg. University of Connecticut Health Center, Farmington, CT, 06030-1110. University of California at Berkeley, USA. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 10, pp. 366-368, October, 1992. Original article submitted April 7, 1992.

 0007-4888/92/0010-1436$12.50 ©1993 Plenum Publishing Corporation

TABLE 1. Action of Gangliosides on Structural and Functional Characteristics of Erythrocyte Membranes in Myocardial Ischemia

Parameter	Animals undergoing mock operation	Myocardial ischemia	Myocardial ischemia 15 min gangliosides
Content of end products of LPO, relative intensity of fluorescence of lipids in a concentration of 1 ml/ml	29.9±5.8	73.8±10.2	42.7±7.5
PEA content (per cent) in outer monolayer of erythrocyte membranes	12.1±1.2	23.8±2.3	10.2±1.1
Na^+, K^+-ATPase activity in erythrocyte membranes, μmoles PI/ml·h	11.23±1.00	6.08±0.56	8.32±0.50

TABLE 2. Changes in Phospholipid Composition of Erythrocytes

Class of phospholipids	Control	Animals undergoing mock operations	Myocardial ischemia 15 min	Myocardial ischemia 15 min + gangliosides 15 min
Phosphatidylcholine	48.4±1.2	49.2±1.2	48.9±1.1	53.9±1.1
Phosphatidylethanolamine	27.8±0.8	24.8±0.8	23.5±0.8	22.5±0.7
Phosphatidylserine	9.8±0.7	8.8±0.6	8.4±0.6	8.3±0.6
Sphingomyelin	12.3±0.9	12.0±0.8	12.1±0.8	11.5±0.9
Phosphatidylinositol	1.7±0.6	1.9±0.5	1.9±0.5	1.2±0.5
Lysophosphatidylcholine	Tr*	3.3±0.7	5.2±0.4	2.6±0.3

*Tr) Trace (less than 0.5%).

in the presence of exogenous Ca^{2+} (2 $mMCaCl_2$). The reaction was stopped with 5 mM EDTA. The Tris-HCl and PLA_2 (*Streptomyces violaceoruber*) were obtained from Sigma, sucrose from Calbiochem, ouabain and Tween-20 from Serva; other reagents were of Russian origin and of the chemically highly pure and pure grades.

RESULTS

The results given in Table 1 show that a significant increase in concentrations of LPO end products took place in the erythrocyte membranes as a result of myocardial ischemia caused by ligation of the coronary artery, as reflected in permeability, structure of the lipid bilayer, and barrier properties of the membrane [12]. It will in fact be seen that myocardial ischemia was accompanied by inhibition of activity of Na^+,K^+-ATPase, a key enzyme controlling active Na^+ and K^+ transport through the membrane. At the same time there was an increase in accessibility of PEA molecules, which are chiefly located in the inner monolayer, to the nonpenetrating chemical hydrophilic modifier TNBS (Table 1), evidence of disturbance of the transbilayer organization of the erythrocyte membranes.

Preliminary intravenous injection of gangliosides inhibited the development of LPO, and the content of its end products under these ischemic conditions was significantly lower than in the absence of gangliosides, and only a little (not significantly) higher than the LPO level in erythrocytes of animals undergoing a mock operation (Table 1). Meanwhile partial restoration of Na^+,K^+-ATPase activity and complete restoration of the normal transbilayer distribution of PEA in the erythrocyte membranes were observed (Table 1).

The results in Table 2 show that myocardial ischemia also was accompanied by changes in the phospholipid composition of the erythrocyte membranes. Marked accumulation of lysophosphatidylcholine (lyso-PC) (5.2%) and a decrease in the PEA concentration took place, whereas there was no significant change in the content of the remaining phospholipid classes. These results reflect the presence of a "stress syndrome" and, as a result, excitation of the adrenergic and pituitary-adrenal systems [3]. As a result the blood level of catecholamines rose sharply, promoting on the one hand their oxidation into adrenochrome and leading to generation of superoxide anion-radicals [2, 3]. On the other hand, interaction of the excess of agonists with β-adrenoreceptors intensified the chain of metabolic conversions of lipids, including successive methylation of PEA, the formation of phosphatidylcholine (PC) from it, and its subsequent hydrolysis by endogenous PLA_2, as a result of which lysophospholipids appeared in the membrane [11]; the latter, like LPO products, can inhibit Na^+,K^+-ATPase activity [13, 14].

Intravenous injection of the gangliosides led to a decrease in the concentration of phospholipid hydrolysis products, namely lyso-PC (2.6%), and to an increase in the PC concentration (Table 2). This may result from the fact

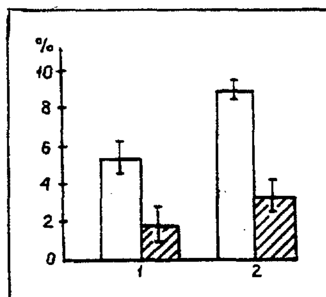

Fig. 1. Action of monosialoganglioside GM_1 on hydrolysis of phospholipids by exogenous phospholipase A_2 in rat erythrocytes: 1) incubation time 5 min, 2) incubation time 20 min. Unshaded columns) Control. Shaded) Preincubation with GM_1 (10^{-8} M). Ordinate, accumulation of lysophosphatidylcholine (in % of total phospholipids).

that gangliosides, on the one hand, cause inhibition of PLA_2 [7], but on the other hand, potentiate transmethylation of PEA [5]; these processes lead to elevation of the membrane PC level. Considering that the possibility of inhibition of PLA_2 activity by gangliosides was demonstrated in model systems on monolayers [7], in the next series of experiments the action of preincubation of the exogenous ganglioside GM_1 with membrane erythrocytes on PLA_2 activity was studied in vitro. As the data in Fig. 1 show, preincubation of the erythrocytes with ganglioside GM_1 led to a significant reduction of PLA_2 activity. We know that PLA_2 activity depends on the Ca^{2+} ion concentration in the medium. It can be tentatively suggested, however, that the observed effect of inhibition of PLA_2 activity by ganglioside GM_1 could hardly be due to the formation of complexes of the gangliosides with Ca^{2+} ions, for the inhibitory effect was exhibited at much lower concentrations of gangliosides than of Ca^{2+} ions (10^{-8} M and 10^{-3} M respectively).

These results illustrate the polyfunctional action of gangliosides in the biomembranes and are evidence of their ability to stabilize not only the lipid bilayer, but also the membrane proteins. The observed effect of the gangliosides can probably be used to prevent damage to biomembranes in pathological states coupled with activation of LPO and PLA_2.

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