CEREBROSIDES AS ONE FACTOR CONTROLLING THE FUNCTIONAL STATE OF THE BLOOD—VESSEL WALL SYSTEM

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In the modern view blood vessels and the blood flowing along them constitute a single system, whose response of vasospasm-hyperaggregation or vasodilatation-hypoaggregation is effected by interconnected mechanisms. Although by now one of the basic mechanisms of regulation of the platelet—vessel wall system has been identified, namely that connected with the prostacyclin—thromboxane system [14], many other factors still await study. Among them attention has been directed to glycolipids, which are essential components of blood cell membranes [11] and can affect both vascular tone [3] and aggregation of blood cells in experiments *in vitro* [4]. In this connection, it was interesting to study the effect of cerebrosides on the functional state of the vascular network and blood cells *in vivo*, for elevation of the blood level of glycolipids and changes in their fractional composition have been demonstrated in some cardiovascular diseases [5].

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 130-150 g, divided into two groups. Rats of the experimental group received intraperitoneal injections of an emulsion of cerebrosides, prepared in a mixture of ethanol and physiological saline in the ratio of 1:20 (v/v), daily for 4 months in a dose of 0.5 ml, equivalent to 5 mg of cerebrosides, per kilogram body weight. Control animals received injections of 0.5 ml of the same mixture but not containing cerebrosides.

The total cerebroside fraction was isolated from bovine brain by Klenk's method and purified on a column with Mark L silica-gel (from Chemapol, Czechoslovakia) [15]. The purity of the cerebrosides was verified by fractionation on a thin layer of Mark G silica-gel (from Sigma, USA) in a solvent system of chloroform—methanol—acetic acid—water in the ratio of 80:13:8:0.3 (v/v), followed by development by sprinkling with a solution of potassium bichromate. Bovine brain cerebrosides obtained from Sigma (USA) were used as the standard.

Aggregation of erythrocytes and platelets and reversibility of endocytosis of the latter were carried out as described previously [2]. Deformability of erythrocytes was determined by passage of the cells through a millipore filter [12].

The state of tolerance of rats to muscular exertion was studied on a model suggested by Avakyan et al. [1]. Arterial blood pressure (BP) was measured by a bloodless method on a BP Recorder (Italy) apparatus. The results were subjected to statistical analysis by the Student—Fisher method.

EXPERIMENTAL RESULTS

Thin-layer chromatography of the total fraction of brain cerebrosides showed (Fig. 1) that the cerebrosides used in the experiments were free from impurities and consisted of three main fractions [6].

Regular daily injections of total cerebroside fraction for 4 months caused a stable rise of BP in rats of the experimental group compared with the control: 137.91 ± 2.97 and 109.4 ± 3.8 mm Hg, respectively ($P < 0.001$). The increase in pulse rate was not significant (440.3 ±

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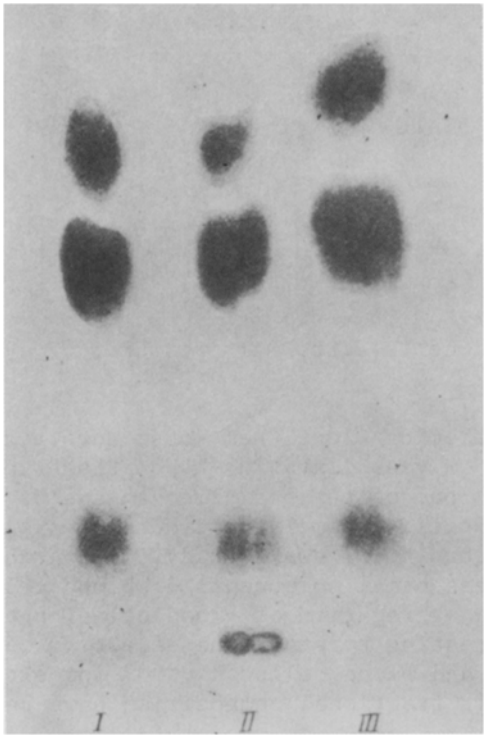

Fig. 1. Thin-layer chromatography of total cerebroside fraction from bovine brain. I) Standard cerebrosides; II) before purification on silica-gel column; III) after purification. Mark G silica gel. Solvent system: chloroform—methanol—acetic acid—water (80:13:8:0.3 v/v).

TABLE 1. Parameters of Platelet and Erythrocyte Function in Rats after Chronic Injection of Cerebrosides in a Dose of 5 mg/kg Body Weight (M ± m)

Group of animals	Platelet aggregation, %	Platelet disaggregation, %	Platelet adhesion, %	Platelet liberation response	Aggregation of erythrocytes, %	Deformability of erythrocytes, %
Control	37,82±2,45 (*n*=11)	61,0±4,34 (*n*=11)	29,54±3,33 (*n*=12)	32,96±4,41 (*n*=8)	36,23±2,33 (*n*=16)	74,01±2,75 (*n*=18)
Experimental	54,58±3,1* (*n*=14)	31,66±5,79* (*n*=15)	54,43±3,73* (*n*=13)	50,74±5,86† (*n*=10)	54,15±2,46* (*n*=19)	54,28±3,34* (*n*=19)

Legend. n) Number of rats in group. *$P \leq 0.001$, **$P \leq 0.05$.

13.57 and 410.3 ± 12.72). Values of parameters of platelet and erythrocyte function in rats after chronic administration of cerebrosides are given in Table 1. Under these conditions, an increase was observed in ADP-induced aggregation, accompanied by a parallel decrease in the degree of their aggregation. An increase also was found in the ability of the platelets to release dye which had accumulated in them, evidence of labilization of platelet secretion processes. Finally, ability of the platelets to undergo adhesion was increased, and this could be a factor increasing the risk of arterial thrombosis.

At the same time, the ability of erythrocytes to aggregate showed a tendency to increase, with a significant decrease in their ability to deform, and this could lead to a disturbance of the rheologic properties of the blood and to disorders of the microcirculation.

The changes mentioned above are important for an understanding both of the long-term increase of vascular tone in arterial hypertension and of the mechanisms of development of vasospasm in the case of a local increase in glycolipid concentration. The increased ability of the platelets to aggregate, discovered in these experiments, may aggravate disorders of vascular tone when physiologically active substances (vasoconstrictors) are released from the platelet granules, and may lead, on the one hand, to the risk of arterial thrombosis and, on the other hand, to blocking of the microcirculatory system by aggregated platelets. This last effect is also related to changes found in the functional state of erythrocytes and, in particular, a decrease in their plasticity [8].

In connection with the results described above, it was decided to study the integrity of the vascular system as a whole in rats after chronic injection of cerebrosides. The onset of muscular fatigue and death of the animals from stress induced by forced dynamic work could act as integral characteristics of this kind. For instance, in rats of the experimental group such stress led to a significant shortening of the time of development both of muscular fatigue (28.0 ± 2.21 min compared with 52.66 ± 2.73 min, $P < 0.001$), and of death of the animals (36.6 ± 2.74 and 66.48 ± 3.95 min, respectively).

Consequently, the increased glycolipid concentration observed in certain cardiovascular diseases can be regarded as a factor with a direct role in mechanisms of circulatory disorders. The increase in hemostatic potential as a result of platelet activation is also related to the pathogenesis of atherosclerosis. Adhesion of platelets in areas of damaged endothelium and their subsequent aggregation, for instance, are regarded by some investigators as a factor triggering atherosclerosis [13]. Investigations [7, 9, 10] which revealed an increase in the concentrations of gangliosides and cerebrosides in sites of atherosclerotic lesions of the human and animal aorta affected by experimental atherosclerosis assume particular importance from this aspect.

The results thus confirm the writers' view that glycolipids are substances of definite interest from the point of view of the study of their possible role in the pathogenesis of cardiovascular diseases.

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