BIOPHYSICS AND BIOCHEMISTRY

Rat Brain Gangliosides during Hypoxia

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The content and composition of rat brain gangliosides in various forms of hypoxia is studied. The content of lipid-bound sialic acid is found to be considerably elevated in the earliest stages of hypoxia combined with hypercapnia and cooling. The content of gangliosides remains elevated over the hypoxia period and returns to control values 48 hours after its discontinuation. Repeated hypoxia results in the same changes in rat brain gangliosides. Hemic sodium nitrite-induced hypoxia also leads to a reliable elevation of these lipids. Hypoxia also causes changes in the content of individual ganglioside fractions: a decreased content of mono- and an increased content of polysialogangliosides.

Key Words: *gangliosides; sodium nitrite; hypoxia; brain; rats*

Despite a large body of investigations and numerous hypotheses, the functional role of gangliosides remains poorly understood in a number of physiological states, among them adaptation to hypoxia of various origin. Taking the above into account and bearing in mind published data on the possible use of gangliosides as adaptogens reducing the severity of damage to nervous tissue [1], we studied the effect of different forms of hypoxia on the total content and composition of rat brain gangliosides. Two forms of hypoxia were studied: hypoxic and hemic. Hypoxic hypoxia develops due to a simultaneous decrease of oxygen and increase of carbon dioxide concentrations, while hemic hypoxia develops after administration of sodium nitrite.

MATERIALS AND METHODS

The model of hypoxic hypoxia with external cooling used by us [2] maximally reproduces the conditions at birth for any warmblooded organism: hypoxia and hypercapnia due to disturbed umbilical circulation and the action of low environmental temperatures. The study was carried out on male unbred white rats weighing 150-200 g. Each animal was placed in a refrigerator (+2-+3°C) in a hermetically sealed 2.4-liter chamber. For assessment of the dynamics of biochemical changes some animals were decapitated after 15, 30, 60, and 90 min and after adynamia was attained. Half of the animals exposed to hypoxia were maintained under normal conditions during 48 hours, after which some animals were decapitated, while others were again exposed to hypoxia. After the repeated exposure to hypoxia the scheme of decapitation was the same: 15, 30, 60, 90 min, and adynamia. Intact animals and animals exposed to hypoxia-hypercapnia at 20°C until they lost the ability to maintain normal posture served as the controls. Hemic hypoxia was induced by subcutaneous injection of sodium nitrite in a dose of 15 mg per 100 g body weight. The animals were examined 10 min after the injection, when they showed signs of acute hypoxia, the blood concentration of methemoglobin then being 11.5%. Gangliosides were extracted from brain tissue (exclud-

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ing the cerebellum) as described earlier [5] with modifications [7]. The content of gangliosides in the obtained extracts was assessed by the content of lipid-bound sialic acid, as described elsewhere [8], and their composition was analyzed by thin-layer chromatography on HPTLC high perfomance plates (Merck) and developed in the following system: chloroform:methanol:0.2% $CaCl_2$ (50:42:11) using a mixture of individual gangliosides (G_{M3}, G_{M1}, G_{D1a}, G_{D1b}, G_{T1b}, G_{Q1b}) as the standard. The ganglioside fractions were stained on plates with Ehrlich reagent and analyzed densitometrically [4].

RESULTS

The total content of gangliosides in the rat brain under various conditions of experimental hypoxia considerably differed from that in the control (Fig. 1). The content of lipid-bound sialic acid per gram of brain tissue reliably increased as soon as after 15 min of the first session of hypoxia-hypercapnia combined with hypothermia. It remained above the control value over the entire period of hypoxia and declined only after adynamia was attained. Two days after the hypoxia session the content of gangliosides in rat brain approached the control level. Repeated hypoxia changed the content of brain gangliosides per gram of tissue in a similar manner. Hypoxia at room temperature also elevated the content of lipid-bound sialic acid per gram of brain tissue in comparison with intact rats. In hemic hypoxia induced by sodium nitrite the content of brain gangliosides 10 min after injection also surpassed the control values.

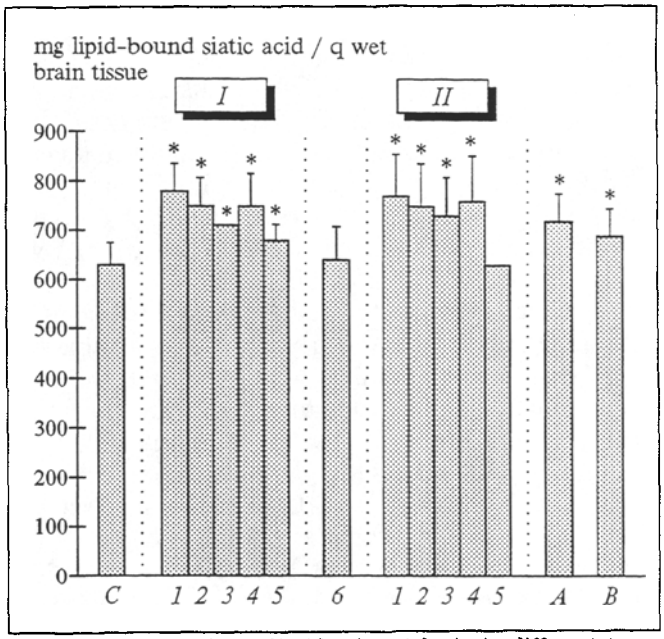

Fig. 1. Content of gangliosides in rat brain in different types of hypoxia (μg lipid-bound sialic acid per g wet brain tissue). *C*) control ($n=25$); *A*) hypoxia+hypercapnia, 120 min ($n=20$); *B*) hemic hypoxia ($n=20$); *I* and *II*) first and second exposure to hypoxia-hypercapnia with cooling: *1*) 15 min, *2*) 30 min, *3*) 60 min ($n=15$), *4*) 90 min, *5*) 120 min ($n=25$), *6*) 48 hours ($n=15$). Asterisk denotes statistical differences ($p<0.05$).

TABLE 1. Content of Gangliosides from Rat Brain in Various Types of Hypoxia

Influence	Ganglioside fraction									
	G_{M3}	G_{M2}	G_{M1}	G_{D3}	G_{D1a}	$G_{T1a}+G_{D2}$	G_{D1B}	G_X	G_{T1b}	G_Q
Control ($n=25$)	trace	–	14.99±0.54	3.62±0.12	44.98±1.10	7.79±1.30	11.75±0.92	3.20±0.76	14.73±0.86	trace
Duration of hypoxia, min										
15 ($n=15$)	trace	–	11.77±0.32	3.81±0.15	35.41±0.68	2.32±0.11	18.33±0.33	1.31±0.29	25.17±0.79	2.27±0.15
30 ($n=15$)	trace	–	12.64±0.52	4.29±0.98	37.93±0.91	6.14±0.14	13.49±1.05	3.61±0.04	20.43±1.02	1.47±0.12
60 ($n=15$)	trace	–	9.23±0.36	4.14±0.38	37.19±0.92	3.82±0.42	13.18±0.63	6.27±0.58	22.23±0.58	0.94±0.10
90 ($n=15$)	trace	–	10.45±0.83	13.19±0.44	41.29±0.84	5.17±0.86	12.70±0.96	2.52±0.47	23.76±1.97	0.72±0.35
120 ($n=25$)	trace	trace	9.78±1.39	1.62±0.18	37.22±1.18	3.43±0.46	18.01±0.76	1.28±0.18	26.63±0.52	1.79±0.07
48 hours after 120-min hypoxia ($n=15$)	0.99±0.45	4.86±0.19	6.81±0.62	1.98±0.28	32.57±0.47	2.74±0.26	18.09±0.68	1.86±0.13	28.23±1.11	1.88±0.28
Duration of repeated hypoxia, min										
15 ($n=15$)	–	–	10.42±0.52	2.26±0.20	35.22±1.38	4.18±0.36	17.71±1.17	2.07±0.28	26.21±1.41	1.96±0.25
30 ($n=15$)	trace	–	8.86±0.84	2.97±0.28	36.67±1.22	4.62±0.49	16.45±1.85	2.39±0.18	25.24±0.68	2.80±0.18
60 ($n=15$)	trace	–	7.87±0.29	1.70±0.31	39.97±0.76	3.67±0.15	16.49±0.36	2.39±0.30	25.75±0.48	1.91±0.14
90 ($n=15$)	–	0.85±0.02	11.34±0.01	1.87±0.07	38.07±0.91	trace	21.68±0.51	1.00±0.21	22.40±1.00	2.85±0.26
120 ($n=25$)	trace	–	8.39±0.30	1.77±0.25	40.14±0.73	3.22±0.73	18.92±1.31	2.13±0.67	24.17±0.47	1.27±0.19
Hypoxia without cooling ($n=20$)	0.74±0.22	0.54±0.10	8.61±0.69	1.00±0.23	38.98±1.01	–	18.95±1.15	–	28.18±0.94	2.98±0.54
Hemic hypoxia ($n=20$)	–	–	10.04±0.20	1.48±0.39	37.71±1.70	2.46±0.59	19.92±0.77	1.03±0.31	25.63±0.93	1.75±0.38

The relative content of individual gangliosides also changed reliably (Table 1): the content of G_{M1} monosialoganglioside and G_{D1a} disialoganglioside increased, while that of G_{D1b} disialoganglioside and G_{T1b} trisialoganglioside increased. These data suggest that the increased content of lipid-bound sialic acid may partially result from sialylation of preexisting glycosphingolipids due to the relatively rapid activation of sialyltransferases.

The reduced content of G_{M1} monosialoganglioside may result from both accelerated synthesis of G_{D1b} disialoganglioside, which may be further sialylated to G_{T1b} trisialoganglioside, and its accelerated catabolism [3]. The fact that the rise in the content of G_{D1b} and G_{T1b} gangliosides considerably surpassed the depletion of G_{M1} ganglioside suggests that the activity of enzymes which take part in its synthesis exceeded that of catabolic enzymes. Increased activity of enzymes of G_{M1} ganglioside catabolism after the discontinuation of adverse influences manifests itself in, on the one hand, the appearance of G_{M2} and G_{M3} gangliosides, products of G_{M1} ganglioside degradation, in the total ganglioside pattern. But, on the other hand, these short-chain monosialogangliosides are precursors of G_{M1} in the rat brain and their appearance 48 hours after the hypoxic session may result from their *de novo* synthesis.

The obtained results suggest that the changes in the content and composition of brain gangliosides in rats subjected to various forms of hypoxia may be compensatory in nature. The increased content of gangliosides and, in particular, of the relative content of polysialogangliosides in the nervous tissue may lead, for instance, to increased rigidity and stability of neuronal membranes [9]. Gangliosides have been previously shown [1] to prevent the oxidative stress-induced inactivation of synaptosome membrane-bound enzymes, and therefore their increased content may facilitate normal functioning of nervous tissue cells during adaptation to hypoxia.

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