MECHANISM OF THE ANTIHYPOXIC ACTION OF LITHIUM HYDROXYBUTYRATE ON EXPERIMENTAL CEREBRAL ISCHEMIA

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The marked antihypoxic effect of γ-hydroxybutyric acid (GHBA) and its sodium salt in various pathological states has attracted the attention of many investigators, and the mechanism of action of sodium hydroxybutyrate has now been largely explained [5]. In 1970 a new compound, namely lithium hydroxybutyrate, was synthesized at the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, and in addition to its powerful antipsychotic action, it also has an antihypoxic action [4].

The aim of the present investigation was to compare lithium hydroxybutyrate and sodium hydroxybutyrate and, in particular, their effects on the basic parameters of brain energy metabolism in total cerebral ischemia.

EXPERIMENTAL METHOD

Brain tissue from male tetrahybrid mice weighing 18-22 g was studied. The brain was removed and frozen in liquid nitrogen after 30 and 60 sec of postdecapitation ischemia. The heads of the control animals were placed in liquid nitrogen immediately after decapitation. The compounds were injected intraperitoneally into the mice in doses of 100 and 250 mg/kg body weight 1 h before sacrifice. The glycogen content was determined by the method in [13], intermediates of glycolysis, adenine nucleotides, and creatine phosphate as in [14], and malonic dialdehyde (MDA) as in [11].

EXPERIMENTAL RESULTS

Postdecapitation ischemia of the brain led to marked activation of glycolysis (Tables 1 and 2), accompanied by a progressive fall in the concentrations of glycogen and glucose-6-phosphate (G6P) in the brain tissue, accompanied by accumulation of lactate. This, in turn, activated the α-glycerophosphate shunt, which is an adaptive mechanism limiting lactate formation, facilitating assemblying of reduced form of NADH, and maintaining the pyruvate and phosphoenolpyruvate (PEP) levels. An increase in the malate concentration in the ischemic brain, as has been suggested [1, 6], is a regulatory response to oxygen deficiency, during which p-malate-oxaloacetic acid performs the role of redox buffer system, promoting oxidation of the accumulated NADH. In addition, accumulation of malate leads to an increase in the concentration of succinic acid, the most effective substrate for energy metabolism during hypoxia [3], in the hypoxic brain. The decrease in the glutamate concentration, combined with an increase in the α-ketoglutarate concentration, is evidence of slowing of the α-ketoglutarate dehydrogenase reaction in connection with a deficiency of oxidized NAD.

Hypoxia led to a progressive decline in the levels of ATP and creatine phosphate, together with an increase in concentrations of ADP, AMP, and free creatine (Tables 1 and 2).

Preliminary administration of GHBA salts to the mice revealed definite differences in their action at the level of glycogen — the principal energy substrate of the ischemic brain: under the influence of sodium hydroxybutyrate the glycogen concentration did not change significantly compared with the hypoxic control, whereas injection of lithium hydroxybutyrate led to a smaller decrease in the glycogen concentration, as could be seen particularly clearly 30

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TABLE 1. Concentrations of Substrates for Glycolysis and Tricarboxylic Acid Cycle in Mouse Brain Tissue after 30 and 60 sec of Postdecapitation Ischemia (in % of concentrations in intact animals)

Experimental conditions	Dose of compound, mg/kg	G6P	α-Glycerophosphate		PEP		Pyruvate		Lactate		Ketoglutarate	Malate	Glutamate	Glycogen	
		duration of ischemia, sec													
		30	30	60	30	60	30	60	30	60	60	60	30	30	60
Ischemia	—	65	69	73	78	73	127	113	143	252	240	231	76	46	19
Sodium hydroxybutyrate	100	56	70	85	100	127	127	127	143	219	160	92	70	45	20
	250	59	76	35	133	136	153	173	149	290	167	85	77	58	25
Lithium hydroxybutyrate	100	53	56	41	94	45	127	40	176	238	167	92	96	84	31
	250	50	61	32	39	36	80	27	168	214	107	115	112	83	29

TABLE 2. Concentrations of High-Energy Phosphate and Creatine in Mouse Brain Tissue after 30 and 60 sec of Total Ischemia (in % of concentrations in intact animals)

Experimental conditions	Dose of compound, mg/kg	ATP		ADP		AMP		Creatine phosphate		Creatine	
		30	60	30	60	30	60	30	60	30	60
Ischemia	—	65	44	203	141	258	258	54	31	128	146
Sodium hydroxybutyrate	100	61	58	206	134	288	200	40	48	147	120
	250	95	48	216	66	296	332	45	44	120	119
Lithium hydroxybutyrate	100	50	27	171	77	400	279	54	42	127	107
	250	63	31	212	82	311	353	28	55	130	92

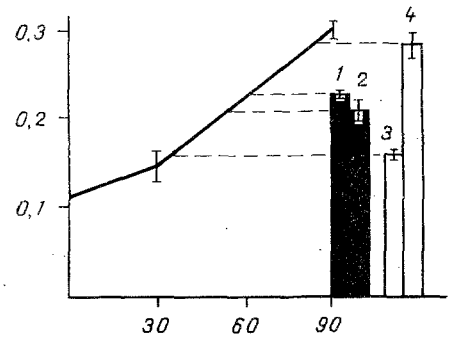

Fig. 1. Effect of sodium hydroxybutyrate and lithium hydroxybutyrate on MDA concentration in mouse brain tissue after decapitation. Ordinate, MDA concentration (in μmoles/mg tissue). Abscissa, time after decapitation (in sec). 1, 2) Sodium hydroxybutyrate in doses of 100 and 250 mg/kg, respectively; 3, 4) lithium hydroxybutyrate in doses of 100 and 250 mg/kg, respectively.

sec after decapitation (Table 1). Later the glycogen concentration in the brain of mice receiving lithium hydroxybutyrate gradually decreased.

No significant difference was observed in the action of the two GHBA salts on the level of principal intermediates of glycolysis against the background of hypoxia, except in the case of concentrations of PEP and pyruvate, which rose considerably under the influence of sodium hydroxybutyrate, especially in the first 30 sec after decapitation. Later, after 60 sec, this effect of the sodium salt of GHBA virtually disappeared (Table 2). Injection of lithium hydroxybutyrate had no such effect.

Sodium hydroxybutyrate, in a dose of 100 mg/kg, normalized the ATP concentration in the brain tissue 30 sec after decapitation (Table 1). Against the background of lithium hydroxybutyrate, no changes of this kind were observed. Both GHBA salts increased the creatine phosphate concentration a little 1 min after decapitation compared with the hypoxic control; no significant differences in their action were observed under these circumstances.

Ischemia for 30 sec led to an increase of 1.3 times in the degree of lipid peroxidation, and ischemia for 90 sec led to an almost threefold increase in the MDA concentration (Fig. 1). Sodium hydroxybutyrate caused some decrease in the degree of peroxidation, and the decrease was dose-dependent in character. Lithium hydroxybutyrate (100 mg/kg) considerably (almost by half) reduced the degree of lipid peroxidation after 90 sec of anoxia, bringing it down to the level observed after 30 sec of ischemia (Fig. 1).

The pathogenesis of cerebral hypoxia is quite complex. One of its principal and initial components is evidently increased release of catecholamines and, as a result, an increase in cAMP concentration [16]. On the one hand, under the influence of cAMP, activation of phospholipase A_2 takes place, and leads to degradation of cell membrane phospholipids, increased vascular permeability, and the development of edema. On the other hand, the accumulating cAMP activates phosphorylase and inhibits glycogen synthetase [15], as a result of which glycolysis is triggered, lactic acid accumulates, and secondary hypoxia develops as the result of swelling of cells of the capillary endothelium in the brain with aggravation of tissue edema.

Activation of lipolysis under the influence of catecholamines, mediated through cAMP, leads to accumulation of unsaturated fatty acids, which are precursors of prostaglandins, which have marked vasoactive properties. Unsaturated fatty acids also act as the original substrates for synthesis of thromboxanes, which promote platelet aggregation and enhanced thrombus formation, and may aggravate hypoxic brain damage. It has been shown that cerebral ischemia causes accumulation, in particular, of arachidonic acid, a precursor in synthesis of prostaglandin E_2 and prostacycline, which in large doses exert a powerful constrictor action on the cerebral vessels [9]. Under these circumstances exposure to hypoxia may lead to a disturbance of the balance between pressor and depressor prostaglandins, with predominance of vasoconstrictor prostaglandins, as is the case, for example, in the ischemic kidney [7].

An important factor in the antihypoxic action of sodium hydroxybutyrate is the normalization of the energy status of the brain tissue, under its influence, through increased production of high-energy compounds (ATP and creatine phosphate). Introduction of Li^+ into the GHBA molecule evidently modifies the mechanism of action of the compound, compared with that of the sodium salt of GHBA. The mechanism of the protective action of lithium hydroxybutyrate may probably be made up of several stages. First, the hydroxybutyrate anion may have a direct action on glycolysis, becoming involved in the Roberts' cycle, and leading to increased formation of succinic semialdehyde, whose protective role in the "hypoxic" brain is well known [3]. Second, it has been shown [8] that lithium prevents release of catecholamines from peripheral nerve endings. If lithium has a similar effect in the CNS, the antihypoxic action of the compound may be realized by inhibition of catecholamine release, with consequent inhibition of cAMP formation. Under these circumstances cAMP-mediated activation of lipolysis is inhibited during hypoxia and the concentration of lipid peroxidation products, notably MDA, which aggravate hypoxic brain damage, is reduced. Third, the inhibitory action of Li^+ on catecholamine release leads to a decrease in activation of phospholipase A_2, and as a result of this, to prevention or, at least, to reduction of cell membrane phospholipid destruction. This, in turn, must bring about a decrease in the liberation of unsaturated fatty acids and prostaglandin formation. During increased release of dihomogammalinoleic acid, a precursor of prostaglandin E_1, which has a vasoconstrictor action on the cerebral vessels, lithium incidentally reduces its concentration, whereas if the concentration of this acid is lowered, lithium raises it [10, 11], and thus plays the role of regulator of the balance between pressor and depressor prostaglandins. This may be particularly important in the ischemic form of cerebral hypoxia, when, as our data [2] showed, lithium hydroxybutyrate is most effective.

With the considerations described above in mice, it can be concluded that lithium hydroxybutyrate is indicated for clinical use in the treatment of hypoxic brain damage.

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CORRELATION BETWEEN NUMBER OF TYPE 2 SEROTONIN RECEPTORS IN THE FRONTAL CORTEX AND INTENSITY OF SEROTONIN-INDUCED HEAD JERKS IN MICE

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Recent investigations have revealed two types of serotonin receptors in the brain: type 1 receptors (S1), binding ^{3}H-serotonin specifically and with high affinity, and interacting with serotonin-sensitive adenylate kinase, and type 2 receptors (S2), highly sensitive to known blockers of serotonin receptors [11, 12, 14]. The functional role of the S1 and S2 receptors is not yet clear [5]. As regards the S2 receptors there is some evidence that they take part in some of the effects of serotonin: in serotonin-induced vasoconstriction of the rat caudal artery [12], in the hypothermic effect of serotonin [3], and in head jerking phenomenon [12, 14] which arises after injection of large doses of 5-hydroxytryptophan (5-HT) and which has been proposed as a test of functional activity of serotonin receptors [7, 13]. It has been shown that antagonists of S2 receptors prevent the development of this form of behavior [14].

Both to understand the functional role of S2 receptors and to assess the importance and informativeness of the head jerking test, it is necessary to know whether any connection exists between the number of S2 receptors and the intensity of this behavioral test. Correlation has recently been shown between the ability of various substances to block S2 receptors *in vitro* and their ability to inhibit the head jerking response in rats [12, 14]. In experiments with repeated electroconvulsions [9] and with chronic administration of antidepressants [6, 8] data have been obtained to show that changes in the number of S2 receptors were associated with changes in the intensity of head jerking. However, data on the character of the effect of antidepressants on the number of S2 receptors are contradictory [8]. Some definite light may be shed on this problem by investigations conducted on intact animals. Our attention was drawn to inbred lines of mice which, as we showed previously, differ in the functional state of the serotonin system of their brain: activity of tryptophan hydroxylase [2] and the concentrations of serotonin and its principal metabolite 5-hydroxyindoleacetic acid [1].

In the present investigation interlinear differences were discovered in the number of S2 receptors in the cerebral cortex and they were compared with the intensity of 5-HT-induced head jerking in mice of inbred lines.

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