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Influence of Changes in Glutathione Concentration on Body Temperature and Tolerance to Cerebral Ischemia

L. S. Kolesnichenko[1]*, V. I. Kulinsky[1], G. V. Sotnikova[2], and V. Yu. Kovtun[3]

[1]Department of Bioorganic and Bioinorganic Chemistry and Department of Biochemistry, Irkutsk State Medical University, ul. Krasnogo Vosstaniya 1, Irkutsk 664003, Russia; fax: (395) 224-0826; E-mail: kulinsky@pp.irkutsk.ru
[2]Department of Physico-Chemical Biology, Irkutsk State University, ul. K. Marksa 1, Irkutsk 664000, Russia; fax: (395) 224-1855; E-mail: gaika@mail.ru
[3]SPC Pharmprotection, Vashutinskoe Shosse 11, Khimki 141400, Moscow Region, Russia; fax: (095) 572-4600; E-mail: pharmg@cityline.ru

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Abstract—Two compounds that deplete glutathione (buthionine sulfoximine and diethyl maleate) with different mechanisms of action decrease body temperature and increase tolerance to complete global cerebral ischemia, both correlating closely with the glutathione concentration decrease. Glutathione apparently participates in the regulations of these functional parameters. GSH diethyl ester does not influence the latter, though it increases moderately the GSH concentration. Injection of GSH ester into the cerebral ventricles or subcutaneously selectively increases the GSH level in the brain and liver. An influence of the brain on the glutathione system in the liver was revealed. Diethyl maleate and GSH ester increase the activity of glutathione metabolizing enzymes under certain conditions.

Key words: glutathione system, compounds depleting GSH, GSH ester, body temperature, cerebral ischemia

Reduced glutathione is involved in many fundamental functions: antioxidative; maintenance of activity of many proteins (enzymes, receptors, etc.); participation in DNA synthesis; proliferation; synthesis of eicosanoids; metabolism of xenobiotics; binding of heavy metal ions; increase in cell resistance [1-3]; and contribution in dualistic redox regulation of gene expression, metabolism, inflammation, and immune responses [4-6]. These molecular and cellular changes must result in functional changes in the body as a whole, but they are studied quite insufficiently. The object of the present work was to investigate the interconnections of the GSH system with body temperature and tolerance to cerebral ischemia. The latter is interesting in view of the special vulnerability of the brain to the action of reactive oxygen species and the influence of cerebral GSH on tolerance of the brain to damage [7-9].

Abbreviations: BSO) L-buthionine [S,R]-sulfoximine; DEM) diethyl maleate; GDEE) GSH diethyl ester; GP_x) glutathione peroxidase; GR) glutathione reductase; GST) glutathione S-transferase.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

One hundred eighty mongrel mice of both sexes having body mass of 18-30 g were used in this work. As biochemical analyzers, substances deliberately changing the GSH level were used: diethyl maleate (DEM) from Sigma-Aldrich (USA); L-buthionine [S,R]-sulfoximine (BSO) from ICN (USA); and GSH diethyl ester (GDEE) synthesized by a method described earlier [10]. Optimal conditions were determined by comparison of injection pathways and study of dose curves and dynamics. All substances were introduced as water solutions: DEM intraperitoneally in 20% (w/v) 2-hydroxypropyl-β-cyclodextrin from Sigma-Aldrich (USA) (solutions in sunflower oil and diethyl sulfoxide gave similar results); BSO introduced into the left lateral cerebral ventricle (in one experimental series, intraperitoneally); GDEE subcutaneously or intracerebroventricularly; corresponding solvents were injected into control mice. GSH concentration and glutathione metabolizing enzymes were determined by spectrophotometric methods [11, 12]. The body temperature was measured by a TPEM-1 electrothermometer in the bowels at the depth 3.5 cm. Tolerance to

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complete global cerebral ischemia was estimated on Lowry's decapitation model by the gasping duration [13]. GSH data were analyzed by *F* and *t* criteria, body temperature by paired *t* test, gasping data (as deviations from normal distribution [13]) by Mann–Witney's *U* criterion; Spearman's correlation coefficients (r_s) and regression equations were computed [14].

RESULTS AND DISCUSSION

Injection of 0.54 mmol/kg BSO intracerebroventricularly and especially 4 mmol/kg DEM intraperitoneally induces marked decrease in GSH concentration on average by 32 and 61%, respectively, in the brain and 56 and 70% in the liver (Table 1) in the absence of any changes of all three glutathione metabolizing enzymes—glutathione peroxidase (GP_x), glutathione S-transferase (GST), and glutathione reductase (GR) (Table 2). The mechanisms of these two GSH-depleting compounds are different: in the first case, GSH consumption is considerably increased (GST conjugates it with DEM); in the second case GSH synthesis is inhibited [1, 15, 16]. However, both substances simultaneously decrease considerably body temperature and increase ischemic tolerance. The identical trend of all these changes is clearly apparent on a "radial" diagram (Fig. 1, a and b), where basal parameter values conform to apexes of a regular polygon, the increase of indices is depicted as "rays", which are prominent beyond the polygon limits, and parameter decrease – as hollows. The coincidence of all the effects of two substances with different mechanisms of GSH decrease gives evidence for the role of this tripeptide in the decrease of body temperature and increase of tolerance to cerebral ischemia.

Table 1. Effect of GSH-depleting compounds and GSH ester on glutathione concentration, body temperature, and tolerance to cerebral ischemia

No.	Series	*n*	Glutathione, μmol/g		Change of body temperature (Δt), °C	Gasping, sec
			brain	liver		
	control					
	ip (CD)	7	2.58 ± 0.17	5.73 ± 0.45	–1.80 ± 0.72	15.5 (15-17)
	icv	17	2.28 ± 0.061	5.85 ± 0.24	–2.04 ± 0.51	16.0 (14-21)
	ip	5	2.14 ± 0.086	5.39 ± 0.31	0.16 ± 0.41	17.0 (16-20)
	sc	9	2.44 ± 0.12	6.19 ± 0.35	–0.42 ± 0.35	16.0 (15-18)
	experimental					
1	DEM (4 mmol/kg, ip, 3 h)	10	1.00 ± 0.073***	1.70 ± 0.22***	–9.08 ± 1.23***	34.0 (17-51)***
2	DEM (4 mmol/kg, ip, 48 h)	5	2.09 ± 0.067*	4.29 ± 0.55	–1.56 ± 0.66	18.0 (16-19)
3	BSO (0.54 mmol/kg, icv, 12 h)	6	1.54 ± 0.086***	2.60 ± 0.46***	–8.73 ± 1.15***	46.0 (27-65)***
4	BSO (0.54 mmol/kg, ip, 12 h)	5	2.15 ± 0.048	4.64 ± 0.84	–1.76 ± 0.44*	17.0 (16-18)
5	BSO (0.25 mmol/kg, icv, 12 h)	11	1.82 ± 0.052***	3.01 ± 0.47***	–6.36 ± 1.26**	30.0 (20-58)***
6	GDEE (56 μmol/kg, icv, 20 min)	9	2.99 ± 0.14***	6.43 ± 0.46	–3.76 ± 1.57	17.0 (15-20)
7	GDEE (2.5 mmol/kg, sc, 2 h)	8	2.35 ± 0.16	7.93 ± 0.41**	–1.35 ± 0.51	19.0 (17-20)*
8	BSO (0.25 mmol/kg, icv, 12 h) + GDEE (56 μmol/kg, icv, 20 min)	11	2.21 ± 0.081	3.69 ± 0.60***	–6.98 ± 1.15***	26.0 (20-37)***
9	BSO (0.25 mmol/kg, icv, 12 h) + GDEE (2.5 mmol/kg, sc, 2 h)	9	1.77 ± 0.058***	4.11 ± 0.43***	–7.31 ± 1.72**	23.0 (20-74)***

Note: In columns 4-6, $\bar{x} \pm s_{\bar{x}}$ values are given; in column 7, medians; in parentheses, deciles (D_1-D_9). Abbreviations: CD, 2-hydroxypropyl-β-cyclodextrin (DEM solubilizer); ip, intraperitoneally; icv, intracerebroventricularly; sc, subcutaneously.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (compared to corresponding control).

Table 2. Effect of GSH-depleting compounds and GSH ester on glutathione metabolism enzyme activities

No.	Series	*n*	GP_x		GR		GST	
			brain	liver	brain	liver	brain	liver
	control							
	ip (CD)	7	30.8 ± 2.31	214 ± 20.8	23.8 ± 1.27	19.0 ± 2.28	67.7 ± 6.78	357 ± 38.7
	icv	11-14	31.9 ± 3.63	178 ± 21.2	30.7 ± 3.25	29.5 ± 2.26	156 ± 12.3	699 ± 86.3
	ip + sc	8	26.4 ± 3.77	193 ± 12.6	36.3 ± 5.58	42.8 ± 2.10	214 ± 16.0	783 ± 78.5
	experimental							
1	DEM (4 mmol/kg, ip, 3 h)	10	28.8 ± 3.40	263 ± 37.7	26.8 ± 1.89	20.3 ± 1.98	78.0 ± 5.12	445 ± 57.2
2	DEM (4 mmol/kg, ip, 48 h)	5	29.4 ± 1.89	208 ± 31.9	38.5 ± 5.43**	35.8 ± 5.17**	158 ± 23.1**	689 ± 34.5***
3	BSO (0.54 mmol/kg, icv, 12 h)	6	36.6 ± 5.03	199 ± 24.0	35.5 ± 2.66	27.0 ± 0.83	191 ± 24.0	679 ± 58.1
5	BSO (0.25 mmol/kg, icv, 12 h)	11	21.6 ± 1.45*	156 ± 19.8	28.5 ± 3.68	28.9 ± 3.91	138 ± 1.38	629 ± 56.8
6	GDEE (56 μmol/kg, icv, 20 min)	9	50.2 ± 4.67**	288 ± 55.0*	39.4 ± 3.34	34.5 ± 3.98	156 ± 15.0	705 ± 80.3
7	GDEE (2.5 mmol/kg, sc, 2 h)	7	40.7 ± 4.95*	291 ± 47.3*	35.7 ± 4.82	35.3 ± 4.28	143 ± 12.1**	655 ± 117
8	BSO (0.25 mmol/kg, icv, 12 h) + GDEE (56 mol/kg, icv, 20 min)	11	44.8 ± 1.71*	291 ± 27.9**	30.7 ± 1.06	36.5 ± 4.27	136 ± 8.20	708 ± 100
9	BSO (0.25 mmol/kg, icv, 12 h) + GDEE (2.5 mmol/kg, sc, 2 h)	9	56.0 ± 9.65***	311 ± 43.0**	31.3 ± 4.22	36.7 ± 4.74	152 ± 24.4	644 ± 96.9

Note: Enzyme activity is expressed in nmol/min per mg protein. Other designations and numbering of experimental series are as in Table 1.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (compared to corresponding control).

Intracerebroventricular injection of BSO at a dose 2.2 times smaller, that is 0.25 mmol/kg, induces similar effects including hepatic GSH decrease (on average 49%). At the same time, injection of 0.54 mmol/kg BSO intraperitoneally decreases body temperature by far less and does not influence significantly GSH concentration in both organs and ischemic tolerance (compare (c) with (b) and (f) on Fig. 1). The studied effects of BSO, which include the GSH level decrease in the liver, are apparently not peripheral, but central effects—they are realized by the brain.

Besides early effects of DEM, it also has a late effect—2 days after the injection when GSH concentra-

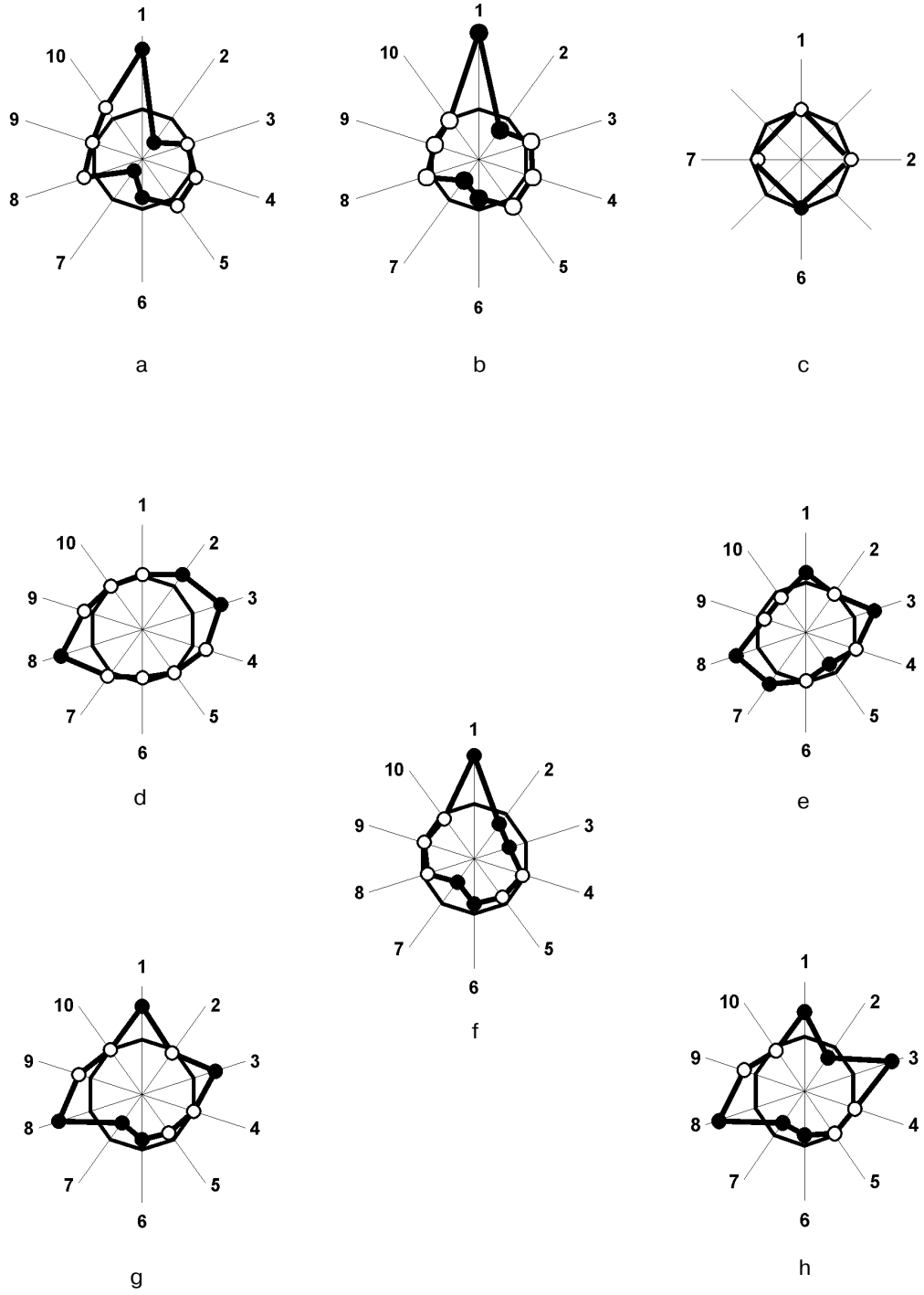

Fig. 1. Changes in biochemical and physiological parameters with deliberate changes in glutathione concentration: a) diethyl maleate (4 mmol/kg, ip, 3 h); b) buthionine sulfoximine (0.54 mmol/kg, icv, 12 h); c) buthionine sulfoximine (0.54 mmol/kg, ip, 12 h); d) GSH diethyl ester (56 μmol/kg, icv, 20 min); e) GSH diethyl ester (2.5 mmol/kg, sc, 2 h); f) buthionine sulfoximine (0.25 mmol/kg, icv, 12 h); g) buthionine sulfoximine (0.25 mmol/kg, icv, 12 h) + GSH diethyl ester (56 μmol/kg, icv, 20 min); h) buthionine sulfoximine (0.25 mmol/kg, icv, 12 h) + GSH diethyl ester (2.5 mmol/kg, sc, 20 min). *1*) Gasping; *2-5*) GSH system in the brain; *6*) body temperature; *7-10*) GSH system in the liver; *2*, *7*) GSH concentration; *3*, *8*) activity of glutathione peroxidase; *4*, *9*) activity of glutathione reductase; *5*, *10*) activity of glutathione S-transferase. •, $p < 0.05$; o, $p > 0.05$.

tion is restored almost to norm a considerable (1.5-2 times) increase in GST and GR (but not GP_x) activity develops in brain and liver (Tables 1 and 2). Induction apparently occurs—the inducibility of these enzymes is well known especially for the liver [15].

GSH esters, including GDEE, are GSH delivery compounds: they increase GSH concentration by penetration into the cells of many organs (but not the brain) and hydrolysis by esterases [10, 15, 17]. We revealed that the influence on GSH level depended on the injection pathways of GDEE: subcutaneously (2.5 mmol/kg) GSH is increased only in the liver, but not in the brain, while in intracerebroventricular injection (in the dose of 56 μmol/kg) GSH is moderately increased only in the brain (on average by 30%). The combination of two pathways of GSH ester injection is a convenient model of selective GSH increase in these two organs. In both pathways GDEE does not influence body temperature, and ischemic tolerance is faintly increased only with subcutaneous injection. In the same series of experiments, GST activity decreases only in the brain. In both series, the GR activity does not change, but GP_x activity increases in the brain and liver (on average by 51-62%). Possibly (especially 3 h after subcutaneous injection) substrate induction of GP_x takes place. The increase in GP_x activity in the liver after GDEE injection not only subcutaneously but also intracerebroventricularly (in the latter case the dose is 45 times smaller) shows the influence of the brain on the GSH level in the liver.

On the background of BSO (0.25 mmol/kg), the cerebral GSH concentration is normalized on GDEE injection into the brain, but not subcutaneously; in both cases decreasing GSH level in the liver and hypothermia are maintained and increased ischemic tolerance remained (medians are lower than on BSO action alone, but differences do not reach statistical significance). In both series, GP_x activity in the brain increases and changes in GST and GR activity are absent. Summing up, in BSO and GDEE combination the main effects of both substances are maintained: specific for BSO the decrease of GSH in the liver and of body temperature and the increase of gasping, specific for GDEE the GP_x activity increase in both organs. Opposed GSH changes in the brain on intracerebroventricular injection are mutually annihilated.

Correlation analysis (Table 3) showed the presence of close interconnections between both the average ($p < 0.01$) and individual data ($p << 0.001$) of all the four studied parameters: positive correlation between GSH concentration in the brain and liver and between GSH concentrations and body temperature, negative correlation between these three parameters and ischemic tolerance. The correlation coefficients are very stable: they change little when the final temperature before investigation is used instead of temperature decrease (Δt^o) and when excluding both series with intaperitoneal BSO injection and all of the control animals. Correlations of functional indices with GSH concentration in the brain are shown in Fig. 2. Regression equations according to individual data for all the animals and to mean values do not differ.

Revealing both single and considerable influence of two GSH depleting compounds, operating by different mechanisms, on body temperature and the gasping duration and the close correlation of these functional parameters with GSH concentration in the brain and liver confirms the importance of GSH in thermoregulation and cerebral tolerance to ischemia. The absence of an influence of GDEE on these parameters can be connected with relatively (in percent of control) smaller action of the used dose on GSH level in comparison with the effects of DEM and BSO. Possibly critical is the considerable decrease in GSH rather than its moderate increase.

It is interesting to compare the importance of the tripeptide for thermoregulation and ischemic tolerance with accumulating data of recent years on direct neuromodulator and neurotransmitter activity of extracellular

Table 3. Correlation of biochemical and functional parameters

Parameter	From individual data ($n = 106$)			
	brain GSH	liver GSH	change of body temperature (Δt), °C	gasping
Brain GSH		+ 0.623***	+ 0.485***	– 0.594***
Liver GSH	+ 0.842**		+ 0.612***	– 0.575***
Δt, °C	+ 0.770*	+ 0.806**		– 0.672***
Gasping	– 0.799**	– 0.815**	– 0.821**	
	from the averages of 9 series and combined control			

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (compared to corresponding control).

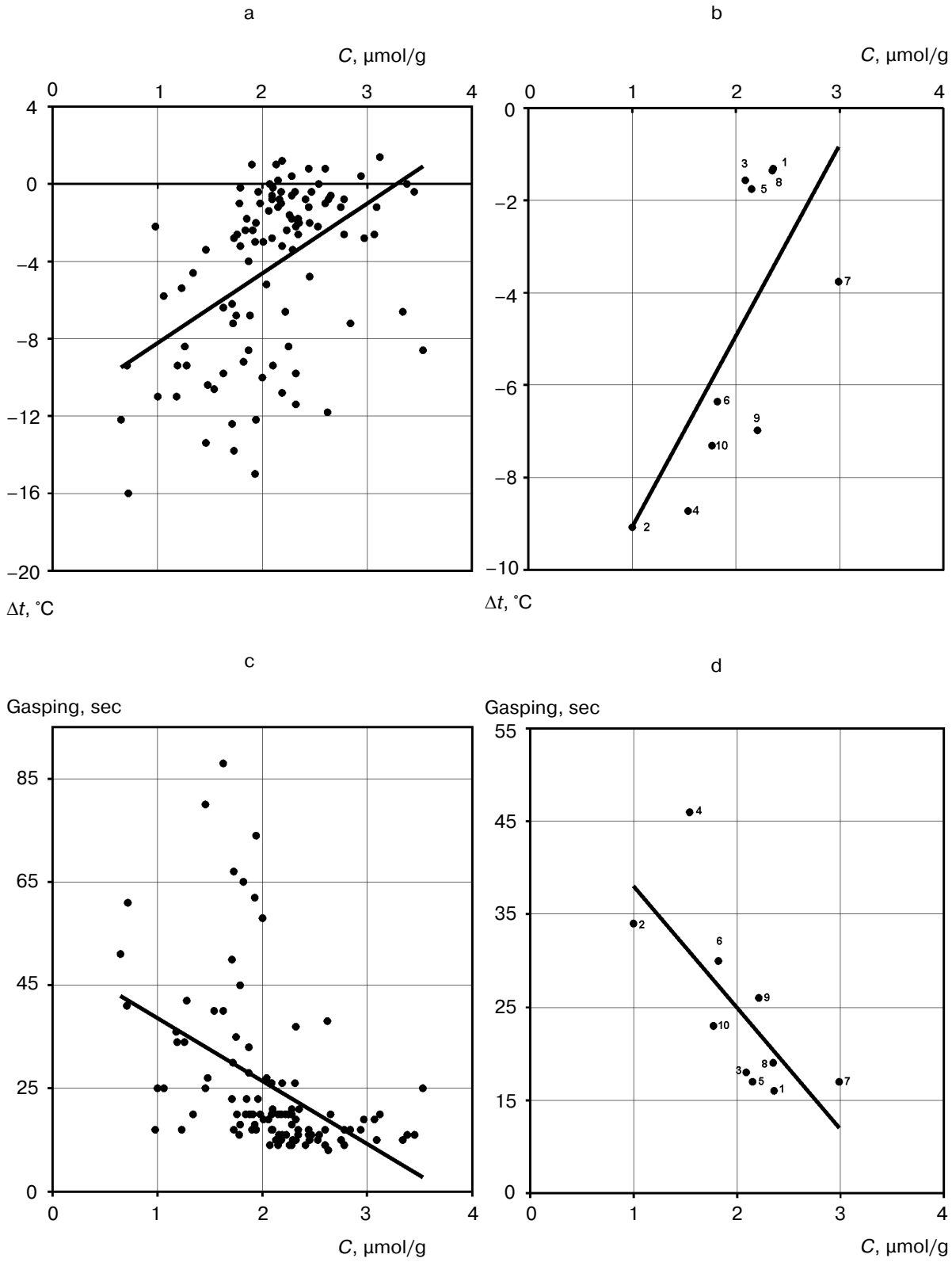

Fig. 2. Correlation of body temperature (a, b) and gasping (c, d) with GSH concentration in the brain. Abscissa, GSH (μmol/g); ordinate, changes of body temperature (°C) (a, b) and gasping duration (sec) (c, d); a, c) individual data; b, d) average data. Denotation of series at (b) and (d): *1*) combined control; *2-10*) numbers of experimental series (sequence of series is the same as in Table 1; in (b) points *1* and *8* coincide). Regression equations: $y = 3.61x - 11.8$ (a), $y = 4.14x - 13.2$ (b), $y = -12.2x + 50.9$ (c), $y = -13.0x + 51.1$ (d).

cerebral GSH, its influence on glutamate receptors, and even on existence in synapses of its receptor sites, which bind GSH [7, 18]. Besides, GSH is a potential reserve of neurotoxic amino acids glutamate and cysteine [7, 8, 19]. In high concentration, they participate in neuronal damage in cerebral ischemia [20, 21].

Different aspects of our results reveal the following facts: 1) BSO in the small dose 0.54 mmol/kg decreases the GSH concentration in the liver only on intracerebroventricular injection, but not intraperitoneally (in the latter the decrease occurs only at doses 3-7 mmol/kg, i.e., on one order of magnitude higher concentration [1]); 2) intracerebroventricular injection of a small GDEE dose increases GP_x activity in the liver in the same degree as intraperitoneal injection of a dose 45 times higher. These facts mean that the brain participates in the regulation of the hepatic GSH system. The mechanisms of these effects are of clear interest.

Finally, DEM increases GST and GR activity in both organs at the later time, and GDEE increases GP_x activity in the liver. This means that these substances in certain conditions not only influence GSH concentration, but increase activity of its metabolizing enzymes.

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