CHANGES IN SORPTION PROPERTIES OF RAT BRAIN LDG PROTEIN AFTER A SINGLE INJECTION OF FORMALDEHYDE

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Changes in the physicochemical properties of proteins in terminal states have been described by many authors [3-5, 7, 8]. The extreme lability of the native structure of a protein, endowing it with its unique specific function, at the same time determines the sensitivity of the protein to harmful factors [12]. In particular, effects of the factors of ischemia, of acute alcoholic poisoning, of γ-irradiation, etc., on the catalytic properties and isozyme spectrum of lactate dehydrogenase (LDH) are known [2, 5, 10, 11, 14]. A study of the character of changes in LDH protein under the influence of these factors may perhaps prove informative for explanation of the mechanisms of development of the corresponding pathological states.

Changes in the sorption properties of this cytosol marker enzyme [15] ied after a single injection of formaldehyde solution, which exhibits protec schemia [6].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats of both sexes weighing 150-250 g and aged from 1 to 3 months. Formaldehyde was injected intravascularly in the form of a 0.2% solution in 0.9% NaCl solution, in a dose of 1.5 μmole/g body weight. The effect of preliminary injection of formaldehyde (immediately before ischemia) was studied under ischemic conditions on a model for small laboratory animals [6] (subgroup *a*), and on animals undergoing a mock operation, with an intact cerebral blood flow (subgroup b). Rats surviving ischemia and the mock operation, without preliminary injection of formaldehyde solution (control 1) and after injection of 0.9% NaCl solution in a volume corresponding to that of the formaldehyde solution injected into animals of the experimental group (control 2) served as the controls. The test objects were the cerebral hemispheres. Samples of brain for analysis were taken from animals undergoing the mock operation at the end of the procedure (control values), at the end of injection of the solutions, and also after 5, 10, 20, 25, 35, and 60 min of ischemia (subgroup *a*) and 5, 20, and 60 min after the operation without ischemia (subgroup b). Activity and the isozyme spectrum of LDH were determined [9] in the postmitochondrial supernatant with sucrose solution. Parallel with sorption on DEAE-Sephadex A-50, sorption also was carried out on DEAE-Sephadex A-25 under the same conditions to study changes in the charge of the LDH protein [1, 13].

The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

No changes in total LDH activity or its sorption properties relative both to DEAE-Sephadex A-25 and DEAE-Sephadex A-50 (activity of the nonabsorbed fraction — ANF) were found in animals with an intact cerebral blood flow after the mock operation or in intact animals. Injection of 0.9% NaCl with the cerebral blood flow intact revealed some increase in total LDH activity. No changes were observed in the sorption properties of LDH after injection of NaCl when the cerebral blood flow was intact.

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TABLE 1. Total Activity (TA) of LDH, Activity of ANF, and LDH_{3+4+5} Activity in Postmitochondrial Supernatant (in IU/ml) of Rat Brain during Ischemia (I) and with Intact Cerebral Blood Flow (II) after a Single Injection of Formaldehyde Solution (M ± m = 6)

Duration of procedure, min	Experimental group						Control 1	
	TA		ANF		LDH_{3+4+5}		TA	
	I	II	I	II	I	II	I	II
0 End of injection of solutions	9,5±0,3*		1,4±0,2*		2,7±0,4		10,5±0,3	
5	11,2±0,5	12,1±0,1*	4,1±0,3	2,3±0,3*	3,5±0,2*	2,17±0,1	10,1±0,4	11,2±0,2
10	9,8±0,2	—	3,5±0,2	—	3,4±0,1*	—	10,2±0,3	—
20	9,5±0,7	10,6±0,3	2,8±0,4	2,1±0,1*	4,1±0,5*	3,5±0,3*	8,6±0,3*	11,4±0,2*
25	8,4±0,4*	—	3,3±0,2	—	3,5±0,3*	—	9,4±0,5	—
35	8,9±0,6*	—	2,6±0,2	—	3,2±0,3*	—	9,0±0,2*	—
60	9,6±0,2*	9,2±0,2*	3,2±0,2	3,9±0,6	3,7±0,3*	4,8±0,2*	9,4±0,3*	10.8±0,2

(Continued)

Duration of procedure, min	Control 1				Control 2					
	ANF		LDH_{3+4+5}		LDH		ANF		LDH_{3+4+5}	
	I	II	I	II	I	II	I	II	I	II
0 End of injection of solutions	3,7±0,5		2,3±0,2		11,5±0,2*		4,6±0,4		2,8±0,2	
5	3,1±0,4	3,1±0,4	3,1±0,3	2,5±0,1	10,2±0,4	12,1±0,4*	3,9±0,4	3,4±0,3	3,9±0,3*	2,4±0,2
10	2,6±0,3*	—	4,3±0,4*	—	9,5±0,3*	—	2,3±0,3*	—	5,4±0,2*	—
20	1,8±0,2*	3,0±0,2	5,4±0,2*	2,8±0,3	9,2±0,4*	11,2±0,3	1,8±0,1*	4,8±0,4	4,9±0,3*	2,3±0,1
25	2,2±0,2*	—	5,3±0,2*	—	8,8±0,4*	—	1,6±0,2*	—	4,6±0,3*	—
35	1,6±0,1*	—	5,2±0,3*	—	8,9±0,3*	—	1,8±0,2*	—	5,5±1,5*	—
60	1,6±0,1*	2,9±0,2	6,0±0,2*	2,8±0,2	9,7±0,4	11,8±0,3*	1,7±0,1*	3,7±0,2	5,5±0,4*	2,3±0,2

Legend. Asterisk indicates significant differences from values in intact brain.

Exogenous formaldehyde caused a significant decrease in total LDH activity and activity of ANF, with the cerebral blood flow intact, and an increase in LDH_{3+4+5} activity.

The creation of ischemia in animals of the control groups led to a decrease in activity of total LDH and ANF and to an increase in LDH_{3+4+5} activity. Injection of formaldehyde before ischemia also led to absence of significant changes in ANF compared with the value of this parameter in the intact brain, and reduced the rate of increase of LDH_{3+4+5} activity compared with that in the unprotected brain. Between 10 and 25 min of ischemia the value of this parameter in the experimental group was 1.2-1.3 times lower, and after 25 min of ischemia it was 1.5-1.7 times lower than in controls 1 and 2 (Table 1).

Thus a single intravascular injection of formaldehyde, with the cerebral blood flow remaining intact, induces a combination of changes in the cytosol marker enzyme LDH, which can be regarded as signs of denaturation changes in the protein. Similar changes are caused by the creation of ischemia in the unprotected brain.

If these results showing changes in the LDH isoenzyme spectrum umder the influence of exogenous formaldehyde, with the cerebral blood flow intact, are compared with analogous data in the literature, obtained by the study of pathological states accompanied by an increased concentration of aldehyde products [2, 5, 8, 11, 12, 15], it can be postulated that aldehydes are involved in the development of this effect, and that formaldehyde influences the state of the LDH isozyme spectrum, which is one system of tissue adaptation to changes in pO_2.

Weakening of the signs of denaturation changes in LDH protein in the presence of exogenous formaldehyde against the background of ischemia is evidence of its ability to protect brain tissue against the denaturing action of ischemia.

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DIFFERENTIAL EFFECTS OF pH, pCO_2, AND PROTEIN CONCENTRATION ON PLASMA CALCIUM ION LEVEL AND ALGORITHM FOR CALCULATING ITS STANDARDIZED VALUE

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Determination of the plasma Ca^{++} concentration, an important parameter of calcium homeostasis in the body, has become possible only recently as a result of the introduction of ion-selective electrodes [2, 7, 11]. To interpret the measured values of the ionized calcium concentration (Ca_i^{++}) they are reduced to pH 7.4 by means of equations obtained by approximating experimental relationships $Ca^{++} = f(pH)$. The equation for pH 7.4 is the one most widely used [12]:

$$\lg \left(Ca^{2+}\right)^{st}_{pH\,7.4} = \lg Ca_i^{2+} - 0.24\left(7.4 - pH_i\right), \quad (1)$$

but it has a number of limitations: It was obtained for average values of concentrations of albumin A = 0.656 mM and total protein P = 37 g/liter [11], and is valid for cases when variation of pH and, correspondingly, of Ca^{++} is due entirely to equilibration of the sample with gas mixtures with different pCO_2 values. Equation (1) is not intended to describe real situations.

This paper describes an attempt to determine the precise algorithm for calculating the standardized calcium $(Ca^{++})^{st}$ by analyzing differential effects of pH, pCO_2, and A(P) of the result of determination.

EXPERIMENTAL METHOD

Heparinized grouped normal plasma (heparin added in a dose of 2 U/ml) was equilibrated in a tonometer (IL-237, USA) with carbogen containing 1.5, 3.9, 5.6, and 7.8% CO_2. The Ca^{++}

*Deceased.

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