COMPARATIVE ANALYSIS OF CEREBRAL CORTICAL PROTEINS OF RATE DIFFERING IN SENSITIVITY TO HYPOXIA

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Adaptation to stress factors, including hypoxia, is effected through activation of nucleic acid and protein synthesis in various tissues and the subsequent development of structural changes in the systems responsible for adaptation [6]. Intensification of protein synthesis during adaptation has been demonstrated for widely different organs and tissues, including the brain [4, 5], and in particular, for the brain structure that is most sensitive to oxygen deficiency, namely the cortex [1, 2]. However, intensification of protein synthesis, determined from the total protein content or the increase in incorporation of label, does not give information on the qualitative and quantitative representation of different proteins in the global picture of the adaptive reaction. No comparative analysis has hitherto been made of the spectra of the protein fractions during hypoxia for a wide range of organs and structures, including the cerebral cortex which, as we know, is distinguished by its high sensitivity to oxygen deficiency The obtaining of such information likewise may be of fundamental importance to the solution of the problem of individual resistance of the organism to hypoxia.

The aim of this investigation was to discover specific proteins in the cerebral cortex whose level of expression changes in response to hypoxia.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 280-320 g, divided beforehand on the basis of their length of survival at a high altitude (11,000 m) into those with high resistance (HR) and low resistance (LR) to hypoxic hypoxia [7]. Some of the animals remained intact (control), others were decapitated 1 h after their ascent in the pressure chamber, and the 3rd group received a course of long-term adaptation to hypoxia, lasting 1 month (the animals were kept for 6 h daily in the pressure chamber at an altitude of 5000 m above sea level for 30 days). On the day after completion of the course of adaptation the animals were decapitated and washed samples of the anterior third of the cerebral cortex (CC) were frozen in liquid nitrogen and kept at −60°C. Subsequent treatment of the specimen and staining of the proteins were carried out as described previously [8]. Two-way electrophoresis in polyacrylamide gel by O'Farrel's method, with certain modifications, was used to fractionate the proteins from CC [3]. Gels from each test were analyzed visually. The relative protein content in the protein fraction examined was estimated by the size and intensity of staining of the corresponding spot, using as the standard for comparison a reference protein topographically nearest to that being estimated, and characterized by stability of the size and shape of the spot, its position on the gels, and the intensity of its staining in all the samples studied. The protein content in the test protein fraction was estimated relative to a reference spot, adopting the following approach: if the content of protein (X) in the spot chosen for analysis was under 1/4 of the protein content in the reference fraction,

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TABLE 1. Relative Protein Content in Labile Protein Fractions of Cerebral Cortex of Rats Differing in Resistance to Hypoxia

Experimental conditions		Mr/pI								
		68/6.1	63/6.15	61/6.4	56/6.2	53/5.9	50/6.1	36/6.2	36/5.9	32/6.4
HR	C	1.00±0.20	0.50±0.20	0.00±0.00*	0.13±0.13	0.70±0.12*	0.50±0.29	0.42±0.20*	1.42±0.20	1.00±0.29
	E	1.13±0.13	0.83±0.11	1.30±0.44*	0.50±0.16	1.63±0.24*	1.25±0.43	1.20±0.25*	1.10±0.40	1.40±0.10*
	A	1.13±0.13	0.75±0.25	0.25±0.19	0.50±0.20	0.92±0.27	1.13±0.13	0.86±0.14	1.21±0.31	0.86±0.14*
LR	C	0.92±0.08	0.92±0.15	0.64±0.21	0.08±0.08*	0.79±0.10*	0.58±0.08*	0.50±0.16	1.88±0.13*	0.56±0.11
	E	1.33±0.17*	0.50±0.16	0.20±0.20	0.67±0.17*	1.50±0.29*	1.00±0.00*	0.50±0.38	1.00±0.42	0.40±0.10
	A	0.75±0.11*	0.42±0.08*	0.42±0.33	0.08±0.08	1.25±0.21	0.67±0.17	0.31±0.13	1.06±0.32*	0.79±0.26

Legend. HR) Animals highly resistant to hypoxia, LR) animals with low resistance to hypoxia; C) control, E) rapid ascent, A) adaptation. Asterisk indicates significant differences ($p < 0.05$). n = 8. For each group.

X was considered to be O. Similarly, at $0.25 < X < 0.75$, X = 0.5; at $0.75 < X < 1.25$, X = 1.0, at $1.25 < X < 1.75$, X = 1.5, and at $X > 1.75$, X = 2; the significance of changes in the protein concentration in the test fractions was assessed by Student's test.

EXPERIMENTAL RESULTS

On the basis of comparative analysis of the gels obtained by electrophoresis of the proteins of CC from animals differing in resistance to hypoxia, an averaged two-dimensional map of the distribution of 339 protein fractions with molecular masses (M_r) of between 20 and 100 kilodaltons and with isoelectric points (pI) within the range 5.4-7.0 was constructed, allowing the experimental material to be standardized between coordinates M_r/pI. On electrophoresis of the proteins of the rat CC a stable localization of over 70% of the protein fractions within the interval pI 6.5-6.9 was characteristic, within which proteins with M_r of 94, 63, 54, 43, 41, and 36 kilodaltons predominated quantitatively. Comparison of the gels for a single sample revealed high reproducibility of the results of fractionation. The most stable protein fractions, whose protein content was unchanged both within the same experimental group and among different groups, were discovered. These protein fractions served in the future as reference proteins.

Meanwhile, from the 339 protein fractions analyzed 15 that showed variability in different versions of the experiment were detected. In nine of these fractions the variability was particularly marked (Table 1). A typical gel, in which areas characterized by the greatest variability are boxed, is shown in Figs. 1 and 2, The value of pI of these fractions was between 5.9 and 6.4, and with respect to molecular weight, three groups could be distinguished among them, with M_r of 32-36, 50-56, 61-68 kilodaltons respectively.

Quantitative and qualitative differences between the variable protein fractions in CC of the HR and LR rats were detected even in the control group. For instance, fraction 61/6.4 was absent altogether from CC of HR rats, but it was represented quite well in CC of LR rats. Traces of protein of fraction 56/6.2 were recorded in CC of both types of animals, but nevertheless the concentration of this protein was twice as high in CC of HR than of LR rats. The concentration of protein of fraction 32/6.4 was significantly lower in CC of LR than of HR rats, but that of fraction 63/6.15, on the contrary, was twice as high (Table 1). Differences are thus found in the initial protein spectra of CC of HR and LR rats.

"Acute ascent" of rats in the pressure chamber to an altitude of 11,000 m, which is a stress factor, led to significant changes in the protein profiles of the rat brain. In CC of HR rats, for example, the protein concentration of six of the nine fractions was increased by 1.5-4 times, with the appearance de novo of significant quantities of a protein with M_r/pI of 61/6.4. In two cases the changes were not significant, and in three cases a small decrease in the protein concentration was found.

In CC of LR rats, after acute hypoxia the formation of any kind of protein de novo could not be detected, but unlike HR, the increase in protein concentration in this case was recorded only for six fractions. The greatest changes affected the initially minor fraction 56/6.2, in which the protein concentration was increased by 8.5 times (Table 1). In seven fractions a significant decrease in the protein content was noted, In this case fraction 61/6.4 initially was not identified in CC of HR rats, but it was present in moderate amounts in CC of intact LR rats. Thus

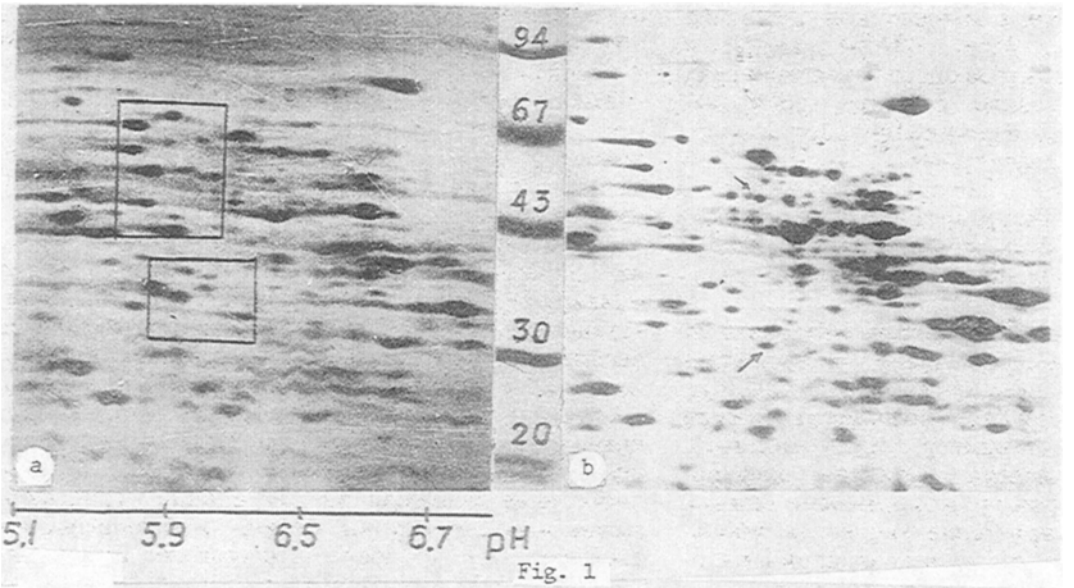

Fig. 1

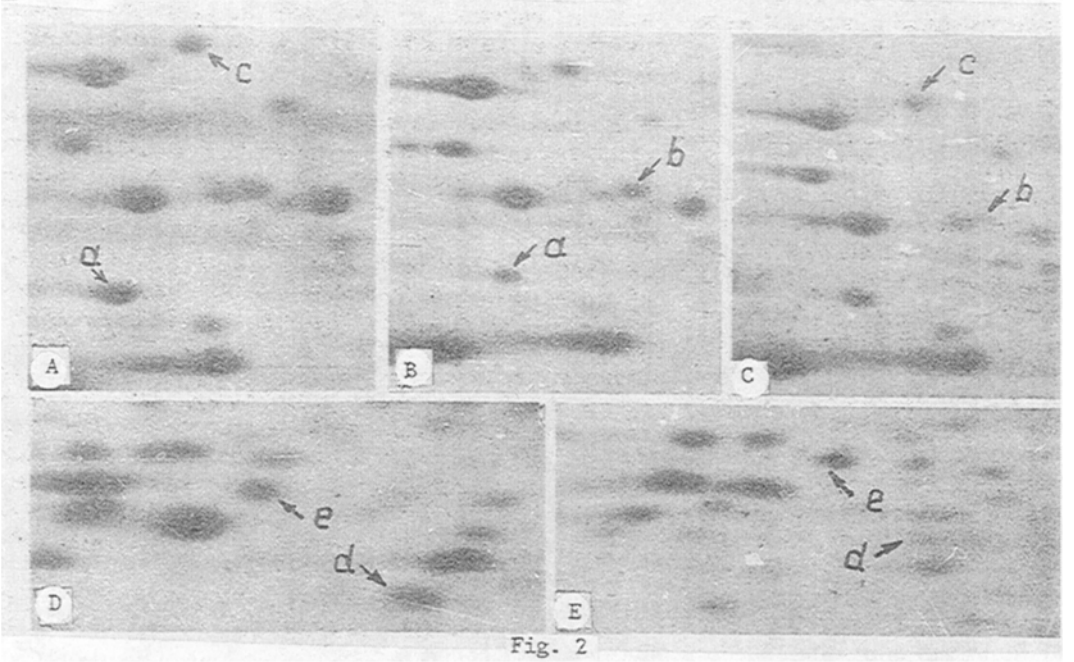

Fig. 2

Fig. 1. Electrophoresis of cerebral cortical proteins from rats with low (a) and high resistance (b) to hypoxia, 1 h after rapid ascent in pressure chamber. Areas characterized by greatest variability of protein fractions are boxed (a); arrows (b) indicate two protein fractions 61/6.4 and 32/6.4, one of which (with high molecular weight) is absent in a and the other is represented significantly less strongly; *a* and b were separated by one-way electrophoresis of marker proteins, listed in order of increasing M_r, and indicated above the corresponding strip in kilodaltons: soy trypsin inhibitor, carboanhydrase, ovalbumin, bovine serum albumim, phosphorylase B. Values of pH gradient in 1st direction plotted along horizontal axis (a).

Fig. 2. Fragments of gels after two-way electrophoresis, taken from Fig. 1A. A, B, C) Within the range of M_r from 42 to 68 kD; D, E) within the range of M_r from 30 to 40 kD; A, D) fragments of gels after electrophoresis of cerebral cortex of a rat highly resistant to hypoxia, 1 h after "acute ascent" in pressure chamber; B, E) the same for control animal, with low resistance to hypoxia; C) the same for rat with low resistance after long-term adaptation. Arrows indicate protein fractions showing significant variability between different experimental groups Arrows a, b, c, d, and e indicate fractions 53/5.9, 63/6.15, 68/6.1, 32/6.4, and 36/6.2 respectively.

the response of the fractions of labile proteins to acute hypoxia did not always coincide in CC of HR and LR rats. As a result of these opposite changes in the two groups the absolute protein content in four labile fractions in CC of LR rats, namely those with M_r/pI of 63/6.1, 61/6.4, 32/6.4, and 36/6.2, was 2-5 times less than in CC of HR rats. For example, for fraction 61/6.4 it was only 15% of the initial level. In all other cases differences in absolute values of protein fractions in CC of the two groups of animals was not significant.

Exposure to acute hypoxia thus leads to rapid transformation of the protein spectrum in CC of both HR and LR rats, appearing as early as after 1 h. In the 1st group of animals, activation of synthesis of labile proteins, and possibly even their formation de novo (appearance of a protein with M_r/pI of 61/6.4), predominates in this case. In the 2nd group of animals, synthetic processes were weaker after hypoxic stress, they were seen in fewer fractions, and protein formation de novo was not found at all. Moreover, in a considerable number of cases synthesis of certain labile proteins was inhibited, or their degradation may even have been intensified. Transformation of synthesis of a group of proteins observed as early as 1 h after exposure to hypoxic stress can be regarded as the realization of emergency compensatory mechanisms under these conditions (emergency mechanisms of adaptation to hypoxia), and which are manifested differently in CC of HR and LR rats, i.e., they are evidently genetically determined.

As a result of long-term adaptation of the rats to hypoxia, changes in the protein profile of the brain involved the same 15 labile protein fractions. The protein content in CC of HR rats in 10 of the 15 fractions exceeded the initial values in intact animals, and in four cases it was below them; it either was equal to values observed after a single exposure to acute hypoxia, or there was a tendency for it to decrease a little (Table 1). By contrast, in CC of LR rats prolonged adaptation to hypoxia led to an increase in protein concentration compared with intact animals in only four fractions: in two fractions it was unchanged and in eight it showed a decrease. Compared with acute hypoxia, in eight of 15 cases a decrease in the protein concentration in the fractions was observed (Table 1). Thus adaptation had an opposite effect on the protein content in the labile fractions of the brain of the two groups of animals. Moreover, the impression was created that changes in protein synthesis in brain tissue are determined by emergency mechanisms of adaptation.

The experimental results indicate that processes connected with transformation of protein synthesis under hypoxic conditions are regulated in CC of HR and LR rats by different mechanisms. This conclusion is in full agreement with our other data showing different ways and unequal efficacy of adaptation of animals differing in their resistance to hypoxia [4]. Analysis of the protein spectra of CC of HR and LR rats also indicated the presence of concrete proteins forming the specific protein portrait of the two groups of animals. Differences found in the abundance of the protein fractions in the brain of HR and LR rats and their different response to hypoxia may reflect the functioning of different genetically determined mechanisms of expression of particular proteins in response to stressful stimulation, including that to acute oxygen deficiency.

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