EFFECT OF HYPOXIA ON THE LACTATE DEHYDROGENASE ISOENZYME COMPOSITION IN THE RAT CAROTID BODY

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The isoenzyme composition of lactate dehydrogenase (LDH) from the carotid body of rats with hypoxic hypoxia and with hypoxia combined with hypercapnia was investigated by electrophoresis in polyacrylamide gel. In response to a decrease in the O_2 concentration in the inspired air there was an increase in the LDH fractions of "muscular type." A change in the ratio between O_2 and CO_2 in the atmosphere affected the character of the change in the LDH isoenzyme spectrum in the carotid body, in agreement with the metabolic theory of chemoreception.

KEY WORDS: isoenzymes of lactate dehydrogenase; carotid body; hypoxia; hypercapnia; chemoreception.

The chemoreceptors of the carotid body are known to participate in the regulation of respiration. Changes in pO_2 and pCO_2 in the arterial blood are independent stimuli of the chemoreceptors [7, 10]. The study of the metabolism of the carotid body is interesting in the light of an understanding of the mechanisms of chemoreception [6, 11]. It has been shown, in particular, that for the carotid body to perform its chemoreceptor function, normal carbohydrate metabolism in its tissue is necessary [1, 2]. A key enzyme in carbohydrate metabolism is lactate dehydrogenase (LDH). Changes in the isoenzyme composition of LDH indicate the pathway along with carbohydrate conversion proceeds. Activation of glycolysis in hypoxia leads to changes in the LDH isoenzyme spectrum by increasing the content of isoenzymes capable of functioning under anaerobic conditions in the presence of high concentrations of pyruvic acid (the "muscular type" of isoenzyme spectrum). This is connected with the intensive synthesis of the M-subunit during prolonged exposure to hypoxia [4]. During brief hypoxia, conformational changes in the protein molecule itself evidently play the decisive role in the change in LDH activity. The possibility cannot be ruled out that lactate and pyruvate participate in the allosteric control of LDH activity [9].

The isoenzyme composition of LDH of the carotid body was investigated in rats with hypoxic hypoxia and a combination of hypercapnia with hypoxia.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 180-240 g. The rat was placed in an exsiccator with a capacity of 4.5 liters for 3-4 h (until death). In the experiments with hypoxic hypoxia the expired CO_2 was absorbed by 30% KOH solution. At the moment of death of the animal the O_2 concentration in the exsiccator was 4.5 ± 0.44% and the CO_2 concentration 0.47 ± 0.09%. In the experiments with combined hypoxia and hypercapnia CO_2 accumulated in the exsiccator. The final concentration of O_2 was 2.15% and of CO_2 16.7 ± 0.36%.* Animals kept under the ordinary conditions of the animal house served as the control.

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TABLE 1. Relative Content of Isoenzyme Fractions of LDH in the Rat Carotid Body

Isoenzymes	Content of LDH fractions (in %)		
	normal	hypoxic hypoxia	hypoxia + hypercapnia
LDH_1	2,7	5,1	3,3
LDH_2	19,3	13,3*	10,6*
LDH_3	35,9	28,4*	31,0*
LDH_4	29,3	34,4	38,5††
LDH_5	12,8	18,8	16,6

*Decrease in LDH fraction ($P < 0.05$).
†Increase in LDH fraction ($P < 0.05$).

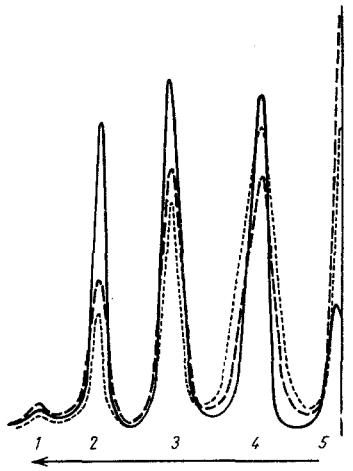

Fig. 1. Isoenzyme spectrum of carotid body under normal conditions and in acute hypoxia. Continuous line – normal; dashes – hypoxic hypoxia; dots – hypoxia plus hypercapnia. Numbers represent serial numbers of LDH fractions.

Proteins of the carotid body were fractionated in a Tris–EDTA–borate system in 7.5% polyacrylamide gel [12]. Electrophoresis was carried out in flat capillary tubes measuring 0.5 × 2.0 × 55.0 mm with a current of 0.2 mA, 180 V, applied to the capillary tube for 45 min. LDH activity was determined histochemically with nitro-BT [8]. The IFO-451 recording microphotometer was used for quantitative evaluation of the results. The significance of differences found was assessed with the aid of the nonparametric criterion X, with a level of significance of 0.05 for a two-sided test [3].

EXPERIMENTAL RESULTS

The results are given in Table 1. They show that during exposure to hypoxia changes take place in the LDH isoenzyme composition, in the direction of an increase in the content of isoenzymes of "muscular type." The composition of the atmosphere (the ratio between the O_2 and CO_2 concentrations) was reflected in the character of the isoenzyme shift (Fig. 1). In hypoxic hypoxia the LDH_2 and LDH_3 fractions were reduced. In combined hypoxia with hypercapnia, fractions LDH_2 and LDH_3 were reduced but LDH_4 was increased. These results suggest that the metabolic response of the carotid body to changes in pO_2 and pCO_2 is specific.

The inequality of the changes in the isoenzyme spectrum of the carotid body in animals exposed to atmospheres of different composition is further evidence in support of the differential function of the carotid body chemoreceptors in the regulation of the pulmonary ventilation.

These results may also indicate that differences in the response of the respiratory system to different stimuli (O_2 deficiency and CO_2 excess) arise actually at the stage of excitation of the chemoreceptors and are not determined entirely by the central formations of the nervous system.

The results are in agreement with those of investigation of the LDH isoenzyme spectrum of rats "elevated" in a pressure chamber [5] and they underline the role of metabolic changes in the mechanism of excitation of the chemoreceptors.

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