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LACTATE DEHYDROGENASE SUBUNIT AND ACTIVITY CHANGES IN HYPERTROPHIED HEART OF THE HYPOXICALLY EXPOSED RAT

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SUMMARY

Rats were exposed to an atmospheric pressure as low as 342 mm Hg (6220 m) for 57 days. This resulted in marked increases in blood hemoglobin concentration and heart weight relative to sea level controls. The lactate dehydrogenase electrophoretic isozyme pattern was visibly altered in extracts from such hearts, showing increased staining density of cathodal components amounting to an 11.8% shift toward greater M-subunit composition. This resulted in such hearts from a more than 2-fold increase in M-subunit, while H-subunit increased only slightly. The sum of both subunit changes produced a 37.5% increase in total cardiac lactate dehydrogenase activity in hypoxically exposed animals as compared to controls.

It has been reported that exposure of rats to simulated altitude results in a decrease in cardiac H/M-subunit ratio, but no change in lactate dehydrogenase (EC 1.1.1.27) activity [1, 2]. In contrast, other investigators found no change in the H/M ratio of lactate dehydrogenase in heart, or in any other organ of animals exposed to hypobaric hypoxia [3]. Thus, the effects of such stress on heart lactate dehydrogenase remain unclear.

Several studies show that the H/M ratio steadily declines in mammalian heart during embryonic and early neonatal development [4, 5]. In this regard, some reports suggest that synthesis of cathodal-migrating lactate dehydrogenase components is closely related to mitotic activity and/or cellular differentiation [6], rather than to tissue oxygen tension and other environmental variables per se. Since heart growth frequently occurs in animals exposed to hypobaric hypoxia resulting in some degree of cardiac hypertrophy [7], it would seem likely that the de-differentiation of cardiac cells occurring during heart growth may play a major role in inducing changes in cardiac lactate dehydrogenase. Yet, in only one of the studies which have examined the effect of simulated altitude on myocardial lactate dehydrogenase [1] were heart-weight data given and in that case cardiac hypertrophy was unexplainably absent.

The present sudy was carried out in an effort to re-examine the effects of a reasonably severe, gradually applied hypoxic stress which is maintained for a period of 2 months on rat heart lactate dehydrogenase, in the presence of accompanying cardiac hypertrophy.

About two dozen male rats of 71 days of age were randomly divided into a

control group and a group to be hypoxically exposed. Mean body weight of both groups was 274 g. Two steel hypobaric chambers (diameter 0.5 m, length 2.0 m) were used for exposure. Initially, internal pressure was set at 447 mm Hg and was then decreased in daily increments of 5 mm Hg over a period of 21 days to give a final pressure of 342 mm Hg. This final pressure, corresponding to a simulated altitude of 6220 m was continued for an additional 36 days, giving a total exposure period of 57 days. Air flow rate through each chamber was maintained at greater than 40 l/min to insure rapid removal of waste gases. Both hypobaric-exposed and control animals were kept in a room at 21–23 °C and were provided with a 12-12-h light-dark cycle. Other than for 15 min each day for carrying out essential maintenance tasks, the hypobaric animals were kept continuously at the atmospheric pressure indicated. Both groups were allowed Purina rat chow and water ad libitum.

Hemoglobin was monitored [8] in blood samples drawn from the tail. Animals were sacrificed by decapitation and the hearts were excised and perfused briefly through the aorta with Krebs–Ringer bicarbonate medium in order to wash out erythrocytes and plasma protein. Lactate dehydrogenase activity and isozyme subunit content were assayed by a previously described method [9]. Cardiac protein content was determined by the method of Lowry et al. [10].

Hypoxic exposure resulted in a large increase in hemoglobin concentration (62%) and marked retardation of body-weight gain (Table I). Heart protein content

TABLE I

EFFECT OF EXPOSING RATS TO A SIMULATED ALTITUDE OF UP TO 6220 m (342 mm Hg) FOR 57 DAYS ON HEMOGLOBIN, BODY WEIGHT AND HEART PROTEIN

Number of animals used in each group indicated by n. Values given as means ± S.E. P values between means are indicated.

Group	Hemoglobin (g/100 ml)	Body wt (g)	Heart protein	
			Measured (mg)	Predicted (mg)
Control (n = 10)	14.49 ± 0.24	524 ± 17	195.2 ± 4.7	196.1 ± 4.2
Hypoxically exposed (n = 11)	23.43 ± 0.45	349 ± 19	241.9 ± 17.1	152.2 ± 4.7
P	< 0.001	< 0.001	< 0.05	< 0.001*

* P value computed for measured heart protein vs predicted heart protein of hypoxically exposed animals.

of the exposed rats was significantly ($P < 0.05$) greater than that of controls, indicating considerable heart growth as a result of the stress. When correction for differences in body weight of the groups is made by predicting [9] what the heart protein content should have been for an equivalent-body weight control animal, it is seen that the heart protein content of the exposed animals had increased even relatively more.

Hypoxic exposure caused an increased staining density of cathodally migrating lactate dehydrogenase isozymes (lactate dehydrogenase-5, etc.) from heart extracts. When quantitated densitometrically this consisted of an 11.8% increase in the per-

centage composition of M-subunit (Table II). This isozyme shift was accompanied by a 37.3% increase in total lactate dehydrogenase activity. From the measured M- and H-subunit activity in the two groups of animals it is clear that both the isozyme shift and increase in lactate dehydrogenase activity resulted primarily from a more than 2-fold increase in the M-subunit. H-subunit increased slightly, but non-significantly.

TABLE II

EFFECT OF EXPOSING RATS TO A SIMULATED ALTITUDE OF UP TO 6220 m (342 mm Hg) FOR 57 DAYS ON LACTATE DEHYDROGENASE ISOZYME COMPOSITION IN M, ACTIVITY OF M- AND H-LACTATE DEHYDROGENASE SUBUNITS AND TOTAL LACTATE DEHYDROGENASE ACTIVITY OF HEART

Number of animals used in each group indicated by *n*. Values given as means ± S.E. *P* values between means indicated.

Group	Lactate dehydrogenase (%M)	Lactate dehydrogenase activity ($in^2 \times 1000$/min per g protein)		
		M-subunit	H-subunit	Total
Control ($n = 10$)	24.30 ± 0.82	3.84 ± 0.21	12.48 ± 0.77	16.32 ± 0.98
Hypoxically exposed ($n = 11$)	36.10 ± 1.25	8.02 ± 0.37	14.39 ± 0.68	22.41 ± 1.05
P	<0.001	<0.001	>0.05	<0.001

These results clearly show that exposure of rats to simulated-altitude oxygen conditions over a period of weeks results in a significant alteration in heart lactate dehydrogenase isozyme pattern. This confirms two earlier studies [1, 2], but stands in disagreement with a third [3]. The present study, however, is the first to report such changes in lactate dehydrogenase during simulated-altitude exposure in the presence of accompanying cardiac hypertrophy. It is also the first study using hypoxically exposed animals to document an increase in lactate dehydrogenase activity and to show that this increase results mainly from a large elevation of the M-subunit. In this regard, it is reported that similar increases in lactate dehydrogenase activity occur in monkey heart cell cultures [11] and in human lymphocytes [12] resulting from increased activities of M-subunit when the lactate dehydrogenase isozyme pattern is altered in favor of more cathodally migrating components.

Considerable uncertainty exists regarding the factor(s) responsible for alterations in lactate dehydrogenase isozyme pattern and the physiological significance of the change. The problems encountered in providing explanations in the latter area have recently been discussed [9]. With respect to the factor(s) responsible for lactate dehydrogenase isozyme change, several studies with kidney showing a parallel increase in M-subunit composition with declining oxygen tension when moving from the cortex to medulla to apex [4, 13] as well as tissue-culture studies [11, 12] have suggested that oxygen tension may exert control over M-subunit synthesis. In addition, it is alleged that other variables may play a role in affecting changes in lactate dehydrogenase, such as hormone titer, denervation [14] and the presence or absence of various metabolic intermediates [15]. All of these environmental variables, however, are also factors which are known to influence rate of cell division and state of cellular differenti-

ation. Some reports suggest that synthesis of the M-subunit and hence the lactate dehydrogenase pattern present in a tissue is related more directly to mitotic activity and state of cellular differentiation than to oxygen tension or other environmental variables [6]. The large number of embryonic and neonatal studies carried out with mammalian heart showing increasing H/M ratio with developmental age [4, 5] could also be interpreted along these lines. Thus, it is quite plausible that the de-differentiation which occurs during rapid cell growth in an adult organ such as the hearts examined here (certainly followed and accompanied by re-differentiation) may have played a direct role in modifying the cardiac lactate dehydrogenase isozyme pattern. That is, the rapid rate of incorporation of new cardiac protein into the enlarging hearts in these hypoxically stressed animals (158.9% of control) presents the possibility that reversion to an earlier ontogenetic myocardial isozyme pattern was simply a normal consequence of rapid organ growth. This being the case, changes in lactate dehydrogenase isozyme pattern may be much less important to preservation of tissue function during oxygen lack than has been assumed over the last decade. It may be of interest that similar changes in lactate dehydrogenase isozyme pattern are observed in hearts enlarged by training to endurance exercise (unpublished observations) and by exposure to CO [16]. In addition, similar changes in both lactate dehydrogenase activity and isozyme pattern occur during the massive cardiac hypertrophy of rats with severe sideropenic anemia [9].

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