Changes in Enzyme Activities in Tissues of Rats Exposed to Hypoxia

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Rats were exposed to various degrees of hypoxia and enzyme activities in their tissues were determined. In general, oxidative metabolism was not increased in response to hypoxia, nor was anaerobic metabolism. Physiological and anatomical changes were concluded to be more important than changes in cellular enzyme activities in the overall adaptation to acute hypoxia.

The physiological responses of laboratory animals to hypoxia are now well characterized, but little work has been reported on the changes in the profiles of tissue enzyme activities under conditions of low partial pressures of environmental O_2 .

Two possibilities for adaptation at the enzymic level have been suggested. First, the studies by Reynafarje (1962) on the changes in cellular respiration and the activity of oxidative enzymes in man suggested that a sufficiently high rate of oxidative metabolism was maintained despite the lowered pO_2 in the capillary blood. Secondly, studies have been made of increases in anaerobic processes that enable tissues to function under conditions of O_2 deficiency (Tappen *et al.*, 1957). To assess the relative importance of these two possibilities and to observe any other enzymic adaptations to the stress, a systematic study of enzyme activities in tissues of rats under various hypoxic conditions was undertaken.

Experimental

Male Wistar rats from the Sheffield University animal colony were used throughout. The animals (approx. 125 g body wt.) were of the same age and were kept in wire-bottomed cages in a controlled environment at 22°C and 45% humidity with 12 h light and dark cycles. Animals were exposed to hypoxia in a specially designed chamber (A. Cryer & W. Bartley, unpublished work), which maintained N_2+O_2 mixtures at atmospheric pressure of any chosen O_2 concentration to within 0.25% O_2 . The temperature and humidity within the chamber approximated the ambient conditions.

Control and experimental animals were allowed water and a commercial pelleted diet [Oxoid modified diet 86; H. C. Styles (Bewdley) Ltd., Bewdley, Worcs., U.K.] *ad libitum*.

Young animals were chosen because of their known ability to survive severe hypoxia better than older rats (Sulkin & Sulkin, 1967), and there were no deaths under the conditions of the following experiments.

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Animals were exposed to hypoxia under three sets of conditions: condition A, for up to 6 days in a 10% O_2 atmosphere; condition B, for up to 16 days in a 12% O_2 atmosphere; condition C, for up to 48 h in a 12% O_2 atmosphere with exercise in a revolving cage requiring the animals to walk 850 m/h. All animals in the experimental groups were compared with controls of the same age, and in addition animals under condition C were compared with animals similarly exercised in air.

Measurements of body, liver, kidney and heart weights were made for both experimental and control animals, and the activities of the enzymes listed in Table 1 were measured in the tissues indicated.

The tissues were prepared for measurement of enzyme activity as outlined previously (Cryer & Bartley, 1973). Glucose 6-phosphatase (EC 3.1.3.9), cytochrome oxidase (EC 1.9.3.1), succinate-cytochrome *c* oxidoreductase (EC 1.3.99.1) and α -glycerophosphate dehydrogenase (NADH) (EC 1.1.1.8) were assayed by the methods given by Cryer & Bartley (1973). Other methods used were: lactate dehydrogenase (EC 1.1.1.27) (Kaplan & Ciotti, 1961); aspartate aminotransferase (EC 2.6.1.1) (Chapman & Bartley, 1968); acyl-CoA synthetase (palmitate-activating enzyme, EC 6.2.1.3) (Pande & Mead, 1968); pyruvate kinase (EC 2.7.1.40) (Taylor & Bailey, 1967).

Results

Changes in body and organ weight. Control animals for all three experimental conditions grew at a rate of 2–3% of their body weight/day, whereas animals under condition A progressively lost weight (–20% of starting weight by day 6) and animals under condition B merely maintained weight. The anorexia known to occur with altitude-induced hypoxia may contribute to these changes (Schnakenberg *et al.*, 1971). Significant weight losses from the livers of experimental animals (all groups) were noted. Kidney weights were unchanged whereas cardiac enlargement (without increase in moisture content) was significant under all the conditions studied. (Student's *t* test was used to compare groups, with at least four

Table 1. *Effect of hypoxic stress on enzyme activities in tissues of the rat*

Animals were exposed to various degrees of hypoxic stress described by conditions A, B and C (see the text) and the enzyme activities in the tissues shown were determined. Activities are expressed as μmol of substrate utilized/min at 25°C . Significant alterations of enzyme activity (per g wet wt. of tissue) are indicated as such when P is less than 0.05; N.S. indicates no significant variation from control. The total numbers of observations at particular times are given in parentheses. Control values are given as means $\pm$ S.D. The maximum percentage change in activity is given for the significant values.

Tissue	Enzyme	Control	Condition A	Condition B	Condition C
Liver	Pyruvate kinase	64.0 $\pm$ 15.0 (9)	N.S. (16)	—	N.S. (12)
	Glucose 6-phosphatase	14.2 $\pm$ 1.1 (6)	N.S. (6)	N.S. (6)	—
	Cytochrome oxidase	15.9 $\pm$ 4.3 (6)	Decreased on days 1 and 5 (5): -38	N.S. (9)	N.S. (12)
	Succinate-cytochrome c oxidoreductase	1.8 $\pm$ 0.4 (4)	Increased on days 1-6 (10): +106	Increased on days 6 and 8 (9): +54	N.S. (12)
	α -Glycerophosphate dehydrogenase	25.6 $\pm$ 4.0 (9)	Increased on days 2, 3, 5 and 6 (13): +58	Increased on days 6, 8 and 12 (7): +62	Increased at 12h and 25h (12): +15
	Lactate dehydrogenase (muscle type)	98 $\pm$ 23 (4)	Increased on days 5 and 6 (16): +40	N.S. (7)	N.S. (12)
	Lactate dehydrogenase (heart type)	56 $\pm$ 17 (4)	N.S. (16)	N.S. (7)	N.S. (12)
	Lactate dehydrogenase (total)	165 $\pm$ 31 (4)	N.S. (16)	N.S. (7)	N.S. (12)
	Aspartate aminotransferase	88 $\pm$ 9 (4)	Increased on days 2-6 (11): +77	N.S. (8)	Increased at 12h and 24h (12): +80
Kidney	Cytochrome oxidase	14.5 $\pm$ 1.5 (6)	Increased on days 5 and 6 (11): +37	N.S. (7)	—
	Succinate-cytochrome c oxidoreductase	3.2 $\pm$ 0.8 (4)	—	Decreased on days 6-14 (7): -52	—
	Lactate dehydrogenase (muscle type)	3.7 $\pm$ 0.6 (4)	N.S. (16)	N.S. (6)	—
	Lactate dehydrogenase (heart type)	40 $\pm$ 3 (4)	N.S. (16)	N.S. (6)	—
	Lactate dehydrogenase (total)	47 $\pm$ 6 (4)	N.S. (16)	N.S. (6)	—
Heart	Cytochrome oxidase	42.8 $\pm$ 18.0 (6)	N.S. (6)	—	Decreased at 12-36h (12): -40
	Lactate dehydrogenase (muscle type)	72 $\pm$ 15 (8)	N.S. (10)	—	N.S. (12)
	Lactate dehydrogenase (heart type)	93 $\pm$ 13 (8)	Decreased on day 1 only (10): -37	—	N.S. (12)
	Lactate dehydrogenase (total)	166 $\pm$ 25 (8)	N.S. (10)	—	N.S. (12)
	Acyl-CoA synthetase	1.5 $\pm$ 0.2 (6)	—	Decreased on days 6-16 (4): -26	Decreased at 24-48h (12): -20
Skeletal muscle	Cytochrome oxidase	8.5 $\pm$ 1.0 (6)	Decreased on days 2-6 (12): -56	Decreased on days 6 and 8 (8): -31	N.S. (12)
	Succinate-cytochrome c oxidoreductase	0.48 $\pm$ 0.10 (6)	—	Decreased on days 6 and 14 (4): -28	N.S. (12)
	α -Glycerophosphate dehydrogenase	11 $\pm$ 3 (6)	—	N.S. (4)	N.S. (12)
	Lactate dehydrogenase (muscle type)	92 $\pm$ 14 (8)	Decreased on days 1, 2 and 5 (10): -62	Increased on days 8, 10, 12 and 16 (8): +49	N.S. (12)
	Lactate dehydrogenase (heart type)	47 $\pm$ 18 (8)	N.S. (10)	Increased on day 16 only (8): +56	N.S. (12)
	Lactate dehydrogenase (total)	140 $\pm$ 24 (8)	Decreased on days 1, 2 and 5 (10): -62	Increased on days 6, 8, 10 and 16 (7): +64	N.S. (12)
	Acyl-CoA synthetase	0.3 $\pm$ 0.1 (6)	—	Decreased on days 6, 8, 10 and 16 (7): -47	N.S. (12)

rats in each group, and significance was ascribed only if P was less than 0.05.)

Changes in enzyme activities in tissues. The control values of enzyme activities were invariant during the experiments (mean values in Table 1). The enzyme activities of the experimental groups were compared with controls killed at the same time by using Student's t test; significance was recorded only when P was less than 0.05.

Summary of changes in enzyme activities in the heart. The hypoxia and the extra work load of the heart under these conditions had little effect on the oxidative metabolism of the organ. Acyl-CoA synthetase activity was consistently lowered under the hypoxic conditions, consistent with the decline in free fatty acid utilization observed in hypoxic isolated rat hearts (Evans, 1964) and in the ischaemic dog heart and its associated triglyceride accumulation (Scheuer & Brachfeld, 1966). This metabolic stability in the heart during hypoxia can be correlated with the ultrastructural findings obtained by Bischoff *et al.* (1969), who concluded that even after chronic (5 months) hypoxia (12% O₂) only moderate changes occurred in the myocardial ultrastructure of the rat, other organs and species being less resistant.

Summary of changes in enzyme activities in the liver. There were no changes in the activities of enzymes associated with glycolysis (lactate dehydrogenase, pyruvate kinase), and the oxidative capacity of the tissue was largely unaffected (cytochrome oxidase, succinate-cytochrome c oxidoreductase). The increases in succinate dehydrogenase activity, also noted by Ainthall & Ramasarma (1969), coupled with the decrease in cytochrome oxidase activity, may reflect the biochemical consequences of the hypoxia-induced disruption of mitochondrial structure noted in the ultrastructural work of Sulkin & Sulkin (1965) and Burgos *et al.* (1970). It would appear that the liver moves to a greater utilization of fat (α -glycerophosphate dehydrogenase) and amino acids (aspartate aminotransferase), as might be expected from the body-weight changes, but the amino acids appear to be oxidized directly and not used for gluconeogenesis, as judged by the unchanged (or in some instances diminished; W. Bartley, E. J. Clegg & V. M. Butler, unpublished work) activity of glucose 6-phosphatase.

Summary of changes in enzyme activities in the kidney. Under conditions A and B the effects of hypoxia on the metabolism of the kidney are negligible.

Summary of changes in enzyme activities in the skeletal muscle. Under the more severe stress of conditions A and B the oxidative metabolism of this tissue was diminished (decreased cytochrome oxidase and succinate-cytochrome c oxidoreductase activities), as was the utilization of fat (decreased acyl-CoA synthetase activity). Small but significant in-

creases in lactate dehydrogenase activity were noted under condition B.

Whole-animal conditions. Condition A. It was calculated from the changes in tissue cytochrome oxidase activity that by day 6 of the hypoxic exposure the body had lost some 23% of its capacity for O₂ uptake, and, since there appeared to be no overall increase in anaerobic metabolism, the energy balance of the animal would suffer. The decline in overall energy-producing processes in the cell must necessarily lead to a disruption of many essential cellular functions, not least protein synthesis, and it would appear that acute adaptation to this condition was at most only partial.

Condition B. In general the observed changes under this condition were smaller in magnitude than those exhibited under condition A, the suggestion being that at 12% O₂ the availability of O₂ for cellular metabolism was more normal than at 10% O₂. This in turn suggests that at 12% O₂ anatomical and physiological adaptations are more adequate for the maintenance of cellular O₂ supply than they are at 10% O₂. The observations here are entirely consistent with the hypothesis that there is a threshold of lowered O₂ concentration above which few distinct biochemical and pathological changes are evident (Fryer, 1969; Bay *et al.*, 1944), this threshold being in the region of 11% in the inspired air.

Condition C. Unfortunately the results obtained under this condition do not present any means of evaluating the adaptations necessary or the reactions made at the cellular level to mild exercise or to mild hypoxic exercise. Neither do they add any information to the study of the cellular reactions to simple mild acute hypoxia. However, the results do indicate the extent to which simple whole-body mechanisms can interact to maintain O₂ and substrate provision for cells in order that they may continue their necessary metabolic functions unimpaired in the face of the stresses applied.

Thus, under the conditions applied in the experiments described here, adaptation to hypoxia is not achieved by an intensification of oxidative processes or a dramatic increase in anaerobic metabolism, and it may be suggested that some of the changes are responses to decreased substrate availability. Biochemical adaptations at the level of tissue enzymes therefore appear to be less important than the anatomical or physiological changes occurring under such stresses.

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