DIFFERENTIAL ALTERATION OF ENZYMES UNDER HYPOBARIA AND HYPOXIA

L. SUSHEELA, A.R. INAMDAR and T. RAMASARMA
Department of Biochemistry, Indian Institute of Science, Bangalore-12, India

Received 26 June 1972

1. Introduction

Animals exposed to hypobaric conditions are subjected to two interacting forces of low atmospheric pressure and decreased partial pressure of the component gases, especially oxygen. The physiological effects obtained thereof are often ascribed to the prevailing hypoxia, although hypobaria is known to elicit specific responses not obtained under hypoxia.

Previous work from this laboratory showed specific increase in activities of hepatic succinate dehydrogenase [1] and tryptophan pyrrolase [2] in rats on brief exposure to hypobaric conditions. The increase in mitochondrial succinate dehydrogenase appeared to be due to increased activity of the existing protein [3] and that of tryptophan pyrrolase in the cytosol to increased amount of the enzyme [4]. These changes probably represent compensatory mechanisms and hence it was of interest to find out whether these enzyme changes were the result of lowered oxygen tension. The results presented in this paper indicate that only the increase in the activity of succinate dehydrogenase and not of tryptophan pyrrolase was obtained under hypobaria. Reduced pressure in some manner was involved in eliciting a specific response in the case of tryptophan pyrrolase, whereas the activation of succinate dehydrogenase was a direct effect of lowered oxygen tension. To the best of our knowledge, this is the first finding of a specific enzyme alteration obtained under hypobaric condition, which is not due to hypoxia.

Abbreviations:
PMS : Phenazine methosulphate
DCIP: 2,6-dichlorophenolindophenol.

2. Materials and methods

2.1. *Exposure to hypobaria and hypoxia*

For hypobaric exposures, male albino rats from the stock colony, weighing 180–200 g were exposed to an atmospheric pressure of 350 ± 5 mm Hg in a decompression chamber. For exposure to hypoxia, animals were exposed to a gas mixture composed of 10% oxygen and 90% nitrogen, having a total pressure of one atmosphere and an oxygen partial pressure equivalent to about half atmospheric pressure at Bangalore, (altitude = 914 metres above mean sea level). At the end of the exposure period, the animals were restored to ambient pressure, killed by stunning and decapitation. The livers were removed and processed further.

2.2. *Succinate dehydrogenase*

The liver (2 g) was homogenized in 10 vol of cold 0.25 M sucrose medium (containing 0.01 M Tris, pH 7.4, and 5 mM EDTA). Mitochondrial fractions were obtained by the conventional differential centrifugation of Schneider and Hogeboom [5]. Succinate dehydrogenase activity (succinate: (acceptor) oxidoreductase, EC 1.3.99.1) was determined spectrophotometrically with PMS–DCIP as electron acceptors as described by Arrigoni and Singer [6]. The basal activity as well as the activity after preincubation of mitochondria with 50 mM succinate [7] was determined.

2.3. *Cytosol enzymes*

The liver (4 g) was homogenized in 1% (w/v) KCl (3 ml/g liver) and the homogenate was centrifuged in a Sorvall RC2B refrigerated centrifuge at 20,000 *g* for 35 min. The supernatant fraction was used for

assaying the enzymes. Tryptophan pyrrolase activity (L-tryptophan: oxygen oxidoreductase, EC 1.13.1.12) was assayed as described by Knox and Auerbach [8]. Tryptophan hydroxylase activity was assayed by the method of Freedland et al. [9] with some modifications as described previously [4]. Tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) was assayed as described by Lin et al. [10]. The animals were always killed around 10 A.M. to avoid changes due to circadian oscillations of tryptophan pyrrolase [11] and tyrosine aminotransferase [12]. Protein was determined by the biuret method [13].

3. Results

The changes in the activities of hepatic tryptophan pyrrolase, succinate dehydrogenase and tyrosine aminotransferase, on exposure of rats to hypobaric and hypoxic conditions are shown in fig. 1. The results indicate that the tryptophan pyrrolase activity increased about 3-fold in the animals exposed to hypobaric stress even for a short period of 4 hr, whereas no change in the activity was observed under hypoxia. On the other hand succinate dehydrogenase activity increased significantly under both the stress conditions. The former enzyme responds specifically to hypobaric stress while the change in the latter is due to lowered partial pressure of oxygen. The observations that succinate dehydrogenase in the partially-activated state in the mitochondrial samples obtained under conditions of hypobaria and hypoxia, could be fully activated on preincubation with succinate implies that the change is qualitative in nature (fig. 1).

It is interesting to note that liver tyrosine aminotransferase and tryptophan pyrrolase, which have the common features of short half-life, circadian oscilla-

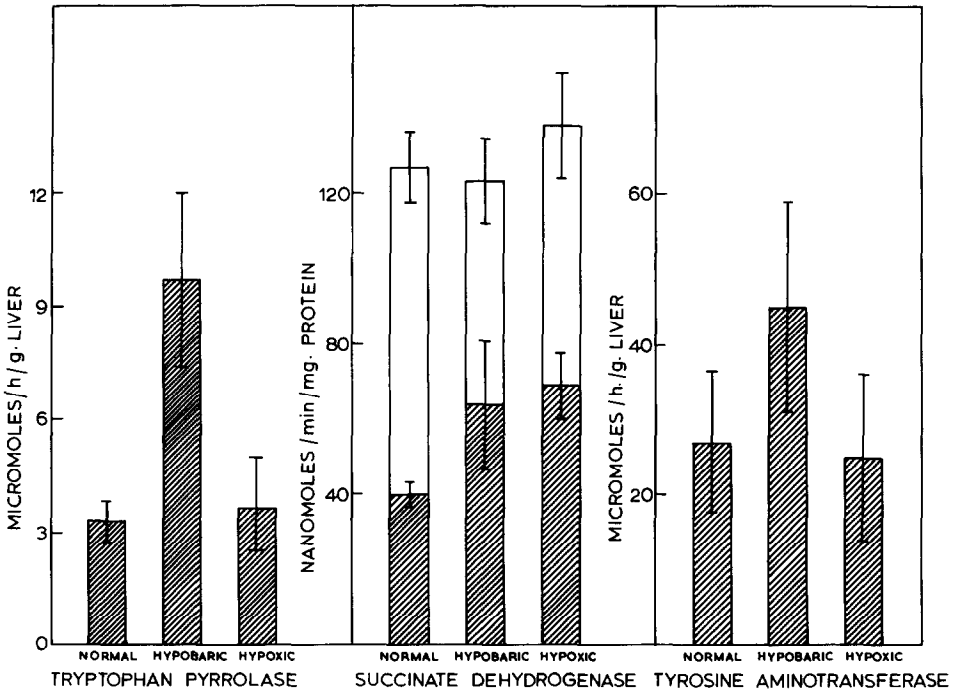

Fig. 1. Effect of hypobaria and hypoxia on the activities of hepatic tryptophan pyrrolase, succinate dehydrogenase and tyrosine aminotransferase. The animals were exposed to these conditions for 4 hr. The values represent mean ± S.D. of independent analyses carried on 6 animals in each group. Tryptophan pyrrolase, significance: controls versus hypobaric $P < 0.01$; succinate dehydrogenase, the shaded blocks represent the basal activity and the unfilled blocks, the excess activity obtained after preincubation of mitochondria at 37° with 50 mM succinate for 7 min, significance: controls versus hypobaric and hypoxic $P < 0.01$; tyrosine aminotransferase, significance: controls versus hypobaric $P < 0.01$.

tions [11, 12] and inducibility by cortisol [14], also increase significantly under hypobaria but not under hypoxia. Tryptophan hydroxylase, the rate-limiting enzyme in the formation of 5-hydroxytryptamine, did not show any change in four hours in both hypobaria and hypoxia (data not given).

4. Discussion

The physiological effects obtained under hypobaria are often ascribed to the prevailing hypoxia. Nevertheless, hypobaria is known to elicit specific responses, not obtained under hypoxia. For instance, reduced pressure has been claimed to lower respiration in yeast [15] which was not obtained with lowered oxygen tension. Exposure of rats to the two conditions of hypobaria and hypoxia was shown to produce opposite effects on the osmotic fragility of red blood cells [16]. The results in this paper also indicate that reduced pressure *per se* elicited specific changes in tryptophan pyrrolase.

The two enzymes selected for study represent two typical examples in oxidative metabolism. Succinate dehydrogenase is a particulate enzyme and the first and rate-limiting enzyme in the succinate oxidase system. Tryptophan pyrrolase is a cytoplasmic soluble enzyme, mainly catabolic in function, utilizes molecular oxygen, accounting for a minor proportion of overall oxygen requirement. Hepatic tryptophan pyrrolase activity is known to be inversely related to concentrations of brain 5-hydroxytryptamine [17] thus influencing mental state. Under limiting oxygen availability, succinate dehydrogenase seems to be modulated for compensation of the lack of oxygen as this forms part of electron transport activities which account for the bulk use of oxygen, yielding energy to the cell. Additional stress of the lowered pressure causes the change in tryptophan pyrrolase.

Acknowledgements

The financial assistance from the Research and Development Organization of the Ministry of Defence, Government of India and in part from Office of Naval Research (U.S.) under contract N00014-71-C-0348 is acknowledged. We thank Mr. S.S. Prakash for technical assistance.

References

[1] H.N. Aithal and T. Ramasarma, Indian J. Exp. Biol. 6 (1968) 179.
[2] A.R. Inamdar, C.K. Ramakrishna Kurup and T. Ramasarma, Biochem. Biophys. Res. Commun. 38 (1970) 220.
[3] H.N. Aithal and T. Ramasarma, Biochem. J. 115 (1969) 77.
[4] A.R. Inamdar, C.K. Ramakrishna Kurup and T. Ramasarma, Biochem. J. 127 (1972) 509.
[5] W.C. Schneider and G.H. Hogeboom, J. Biol. Chem. 183 (1950) 123.
[6] O. Arrigoni and T.P. Singer, Nature 193 (1962) 1256.
[7] T. Kimura, J. Hauber and T.P. Singer, J. Biol. Chem. 242 (1967) 4987.
[8] W.E. Knox and V.H. Auerbach, J. Biol. Chem. 214 (1955) 307.
[9] R.A. Freedland, I.M. Wadzinski and H.A. Waisman, Biochem. Biophys. Res. Commun. 5 (1961) 94.
[10] E.C.C. Lin, B.M. Pitt, M. Civen and W.E. Knox, J. Biol. Chem. 233 (1958) 668.
[11] M.I. Rapoport, R.D. Feigin, J. Burton and W.R. Beisel, Science 153 (1966) 1642.
[12] R.W. Fuller, Proc. Soc. Exptl. Biol. Med. 133 (1970) 620.
[13] A.G. Gornall, C.J. Bardawill and M.M. David, J. Biol. Chem. 177 (1949) 751.
[14] R.T. Schimke and D. Doyle, Ann. Rev. Biochem. 39 (1970) 929.
[15] S.F. Cook, J. Gen. Physiol. 14 (1931) 55.
[16] A.T. Bernardini, Federation Proc. 28 (1969) 1165.
[17] A.R. Green and G. Curzon, Nature 220 (1968) 1095.