Pressure and Lactate Dehydrogenase Function in Diving Mammals and Birds
Author(s): Donald A. Croll, Michele K. Nishiguchi, Sandor Kaupp
Source: *Physiological Zoology*, Vol. 65, No. 5 (Sep. - Oct., 1992), pp. 1022-1027
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/30158556
Accessed: 30/04/2010 16:27

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at http://www.jstor.org/page/info/about/policies/terms.jsp. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at http://www.jstor.org/action/showPublisher?publisherCode=ucpress.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

The University of Chicago Press is collaborating with JSTOR to digitize, preserve and extend access to *Physiological Zoology.*

http://www.jstor.org

Pressure and Lactate Dehydrogenase Function in Diving Mammals and Birds

Donald A. Croll[1,*]
Michele K. Nishiguchi[2,†]
Sandor Kaupp[2]
[1]Physiological Research Laboratory A-004, Scripps Institution of Oceanography, La Jolla, California 92093; [2]Marine Biology Department A-002, Scripps Institution of Oceanography, La Jolla, California 92093

Accepted 4/21/92

Abstract

*Emperor penguins (*Aptenodytes forster*), elephant seals (*Mirounga angustirostris*), and sperm whales (*Physeter catodon*) have been shown to dive to considerable depths (265 m, 1,500 m, and 1,140 m, respectively). These animals must cope with extreme changes in hydrostatic pressure as they dive. The effects of hydrostatic pressure on the Michaelis-Menten constant,* K_m*, of cofactor binding of NADH of muscle* M_4 *(muscle type) lactate dehydrogenase (LDH) was measured for these diving vertebrates and compared with a nondiving mammal, the domestic rabbit. No effect of pressure changes as great as* 2.066×10^4 *kPa (204 atm) was observed in either the diving or nondiving species LDH preparations. Results support the hypothesis that, at least concerning the* K_m *of NADH in the* M_4 *LDH of the diving vertebrates examined, the LDHs of warm-blooded divers do not appear to be affected by changes in hydrostatic pressure and the enzyme may be preadapted for insensitivity to pressure perturbations.*

Introduction

The deepest recorded dive depths for pinnipeds, cetaceans, and birds have been found in elephant seals (*Mirounga angustirostris*), sperm whales (*Physeter catodon*), and emperor penguins (*Aptenodytes forsteri*), respectively. DeLong and Stewart (1991), using recording devices, found that elephant seals can dive as deep as 1,529 m; sperm whales have been found

* Present address: National Marine Fisheries Service, National Marine Mammal Laboratory, 7600 Sand Point Way NE, Seattle, Washington 98115.
† Present address: Biology Department, 225 Sinsheimer Laboratories, University of California, Santa Cruz, California 95064.

Physiological Zoology 65(5):1022–1027. 1992. © 1992 by The University of Chicago.
All rights reserved. 0031-935X/92/6505-9204$02.00

entangled in deep-sea cables at depths of 1,140 m (Heezen 1957); while Kooyman et al. (1971), using capillary depth gauges, found that emperor penguins dive to 265 m. When diving to these depths these animals must cope with extreme changes in hydrostatic pressure.

Hydrostatic pressure has been shown to have a pronounced effect on the biochemical function of enzymes and transport proteins in several shallow-living invertebrates and teleost fishes (see review by Siebenaller and Somero [1989]). Hydrostatic pressure may perturb protein and membrane function through a number of mechanisms where changes in volume may be involved such as: (1) ligand binding affinity, (2) catalytic rates, (3) structural stability, and (4) membrane fluidity (Somero 1990). Such changes in membrane protein function have been suggested as a mechanism for a high-pressure nervous syndrome (MacDonald 1984). These changes can also lead to perturbations in metabolic rate via perturbations in rates of enzymatic catalysis, ion transport, and hormone, neurotransmitter, and neuromodulator binding (Siebenaller and Somero 1989). Siebenaller (1984) studied the effect of pressure on the Michaelis-Menten constant, K_m (the substrate concentration at ½ maximum reaction velocity), of cofactor binding of NADH of muscle M_4 lactate dehydrogenase (LDH) in shallow- and deep-living fishes. He found that K_m values doubled under pressures of 68 times surface pressure (equivalent to approximately 680 m in depth) at 5°C in shallow-living fishes. Deep-living fish showed little change in K_m values at high pressure. The maintenance of a constant K_m in the face of environmental change is critical in maintaining the proper catalytic rate and regulatory sensitivity (Hochachka and Somero 1984). Because pressure effects K_m function in fishes, we decided to study the effects of pressure on the K_m of NADH muscle M_4 LDH in diving mammals and birds that experience significant changes in hydrostatic pressure as they dive.

Maintenance of M_4 LDH function is particularly interesting because, as breath-hold divers, these vertebrates have been shown to accumulate significant concentrations of lactate during diving (see Kooyman 1989 for a review). The metabolism of lactate, therefore, is important in the maintenance of homeostasis in these organisms. Perturbations in LDH function associated with pressure changes could lead to significant changes in metabolic function at depth. Lactate dehydrogenase is a key enzyme in determining the capacity for anaerobic exercise in an animal because of its importance in locomotory muscle function. Changes in LDH function could result in changes in ability to capture prey, ability to escape predation, or overall metabolic rates. By examining the effects of pressure on the rate-limiting step of LDH function, affinity of NADH, the importance of pressure-

induced perturbations in enzyme function in warm-blooded diving vertebrates should begin to emerge.

Material and Methods

Purification of LDH

Purified rabbit M_4 LDH was obtained from Sigma Chemical, Saint Louis. All tissue samples were taken from adult animals and stored at −30°C prior to analysis. Elephant seal muscle was taken from the latissimus dorsi, sperm whale muscle from the cervical region, and penguin muscle from the pectoralis. Purification of elephant seal, sperm whale, and emperor penguin LDH was modified from a procedure taken from Yancey and Somero (1978). Tissue was prepared by grinding 1 vol tissue with 2–3 vol 50 mM potassium phosphate buffer, pH 6.8, and the homogenate centrifuged at 14,000 rpm for 30 minutes. A 1:1 ratio of 50 mM potassium phosphate, 1 M KCl buffer, pH 6.8, was added to the supernatant. For each microliter of supernatant, 0.07 mL of 0.15 mM NADH solution was added. The homogenate was then purified through oxamate-Sepharose affinity chromatography. The column was equilibrated with 50 mM potassium phosphate, 0.5 M KCl buffer, pH 6.8. The crude homogenate was added to the column, followed by 1 column volume of buffer containing 50 mM potassium phosphate, 250 mM KCl, and 200 μm NADH, pH 6.8, to insure binding. A 50 mM potassium phosphate, 250 mM KCl, 2,000–2,500 μM NAD^+ solution was added to elute only the M_4 LDH isozyme. Fractions were collected and monitored for LDH activity. A final elution buffer consisting of 50 mM potassium phosphate, 250 mM KCl, pH 6.8 (no cofactor), was used to elute other isozymes of LDH. Isozyme purification was confirmed using native polyacrylamide gel electrophoresis and LDH activity stain.

Lactate Dehydrogenase Enzyme Activity Assay

All samples were assayed in 80 mM imidazole buffer, 100 mM KCl, 5 mM pyruvate at pH 7.0 at 37°C. We used NADH concentrations of 120, 100, 80, 60, 30, and 15 μM to obtain the K_m. Samples were assayed at either 101.3 kPa (1 atm) or 2.066×10^4 kPa (204 atm), with a Lambda 3B Perkin-Elmer spectrophotometer equipped with thermal regulation. A mineral oil pump was used to produce high pressure in a specially modified pressure chamber for assays. All assays were monitored at 340 nm at 37°C. Two determinations of K_m were made for each species with Wilkenson nonlinear regression.

Results

Results of K_m analysis are plotted in figure 1. In all warm-blooded vertebrates studied, there was no effect of pressure on the K_m of NADH at 37°C. Michaelis constant values ($K_m \pm$ SD) for each species at the two pressures were as follows: rabbit 101.3 kPa (19.9 ± 2.1), 2.066 × 10^4 kPa (18.0 ± 1.7); emperor penguin 101.3 kPa (34.2 ± 4.16), 2.066 × 10^4 kPa (34.2 ± 8.5); elephant seal 101.3 kPa (32.0 ± 6.1), 2.066 × 10^4 kPa (29.1 ± 1.9); sperm whale 101.3 kPa (29.2 ± 9.8), 2.066 × 10^4 kPa (28.2 ± 10.4).

Discussion

In all divers studied we found no significant effect of pressures up to 2.066 × 10^4 kPa at 37°C on muscle M_4 LDH K_m of NADH. The pressure examined, 2.066 × 10^4 kPa (204 atm), was greater than that encountered by any of the study animals. This result is in contrast to fish, in which a twofold increase in the K_m of NADH is seen for M_4 LDHs of shallow-water fishes at 5°C

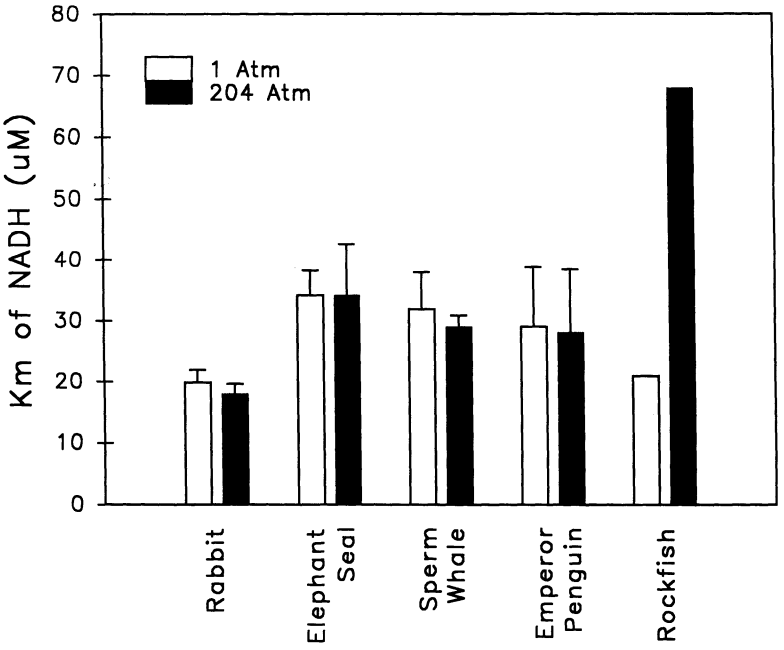

Fig. 1. The effect of pressure on the K_m *of NADH for* M_4 *muscle LDH in deep-diving marine mammals and birds. Bars represent mean ± SD. Rockfish data are from* Sebastolobus alascanus, *a shallow-living rockfish (Siebenaller and Somero 1989).*

(Siebenaller and Somero 1979). Thus, in at least the parameter we measured, there does not appear to be a significant change in LDH function as a result of 200 atm of pressure.

Two hypotheses may account for our observations: (1) because diving mammals and birds experience such wide changes in pressure, they may have evolved specialized enzyme systems that are insensitive to changes in pressure; and (2) diving mammals and birds have enzymes similar to non-divers, but these enzymes are insensitive to changes in pressure. Comparison with the rabbit at 37°C would seem to indicate that the second hypothesis is more likely. We found that pressure had no effect on the K_m of NADH in rabbit muscle M_4 LDH, which was similar to our observation for the diving vertebrates. Thus, insensitivity appears to be a preadaptation for diving in birds and mammals.

A major difference between the fish and diving mammal and bird data is the temperature at which the enzymes normally function in vivo. The fish studied by Siebenaller and Somero (1979) are normally found at temperatures around 5°C. In contrast, the body temperature of the animals we studied is maintained around 37°C. Only small changes in habitat temperature are needed to result in evolutionary changes in the primary structure of enzymes (Hochachka and Somero 1984). The LDH of warm-blooded vertebrates may have a less fluid structure that allows it to function at higher temperatures. Changes in the amino acid sequence of LDH necessary to maintain proper catalytic function at higher temperatures in warm-blooded animals may have also led to pressure insensitivity. Regardless of the mechanism it appears that, at least concerning the affinity of NADH in the M_4 LDH, these warm-blooded divers are preadapted and need no specialized adaptation in protein structure to cope with the pressures they experience routinely.

Acknowledgments

Dr. George N. Somero provided considerable guidance in experimental design, execution, and analysis. In addition, he provided technical assistance and insightful comments on the manuscript. Dr. John Heyning of the Los Angeles County Museum of Natural History, Phillip Thorson of the University of California, Santa Cruz, and Dr. G. L. Kooyman of the Physiological Research Laboratory, Scripps Institution of Oceanography, kindly provided tissue samples for analyses. We thank the students and staff of the Somero laboratory for their patience and assistance in laboratory analyses. D. A. Croll was supported by United States Public Health Service National Institutes of Health grant HL 17731 to Kooyman. M. Nishiguchi was supported

by Office of Naval Research contract 1000014-87K-0012 to Dr. G. N. Somero. Additional support was provided by the Asociacion de Biologos Ambulantes.

Literature Cited

DeLong, R. L., and B. S. Stewart. 1991. Diving patterns of northern elephant seal bulls. Mar. Mammal Sci. 7:369–384.

Heezen, B. C. 1957. Whales entangled in deep-sea cables. Deep-Sea Res. 4:105–115.

Hochachka, P. W., and G. N. Somero. 1984. Biochemical adaptation. Princeton University Press, Princeton, N.J.

Kooyman, G. L. 1989. Diverse divers. Springer, Berlin. 200 pp.

Kooyman, G. L., C. M. Drabek, R. Elsner, and W. B. Campbell. 1971. Diving behavior of the emperor penguin, *Aptenodytes forsteri.* Auk 88:775–795.

MacDonald, A. G. 1984. The effects of pressure on the molecular structure and physiological functions of cell membranes. Philos. Trans. R. Soc. Lond. 304B:47.

Siebenaller, J. F. 1984. Structural comparison of lactate dehydrogenase homologs differing in sensitivity to hydrostatic pressure. Biochim. Biophys. Acta 786:161.

Siebenaller, J. F., and G. N. Somero. 1979. Pressure-adaptive differences in the binding and catalytic properties of muscle-type (M_4) lactate dehydrogenases of shallow and deep living marine fishes. J. Comp. Physiol. 129:295–300.

———. 1989. Biochemical adaptation to the deep sea. Aquatic Sci. 1:1–25.

Somero, G. N. 1990. Life at low volume change: hydrostatic pressure as a selective factor in the aquatic environment. Am. Zool. 30:123–135.

Yancey, P. H., and G. N. Somero. 1978. Temperature dependence of intracellular pH: its role in the conservation of pyruvate apparent Km values of vertebrate lactate dehydrogenase. J. Comp. Physiol. 125:129.