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Biochemical indices of high pressure tolerance in marine mammals

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13. ABSTRACT *(Maximum 200 words)*

The metabolic effects associated with the dramatic and repeated hydrostatic pressure changes faced by marine mammals are unknown. By measuring glucose utilization and lactate production, the effect of 2000 psi of pressure on glycolysis in red blood cells was compared among marine and terrestrial mammals. The effect of pressure on the kinetics of lactate dehydrogenase in cardiac tissue of marine and terrestrial mammals was also evaluated. Pressure affected LDH kinetics similarly in both groups, causing no change in V_{max} or K_m for pyruvate or NAD^+, a decrease in lactate K_m and an increase in NADH K_m. At pressure, marine mammal RBCs generally had little change in glucose utilization rates relative to terrestrial mammals, in which the rate decreased. Lactate production rate was enhanced in some marine mammals, remained relatively unchanged in others and generally decreased in terrestrial mammals. Lactate/glucose was well below the theoretical value of 2.0 except for dolphins and humans. In most cases, lactate/glucose shifted to higher values under pressure, suggesting a shift in metabolic pathway toward glycolysis. The effect of pressure on glycolysis is apparently complex, involving individual enzymes, possibly a shift in metabolic pathway and possibly glucose transport.

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hydrostatic pressure, lactate dehydrogenase, glycolysis, RBC, erythrocyte, marine mammal

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FINAL TECHNICAL REPORT

GRANT #: N0014-93-1-0457

PRINCIPAL INVESTIGATOR: Dr. Michael A. Castellini

INSTITUTION: University of Alaska, Fairbanks

GRANT TITLE: Biochemical indices of high pressure tolerance in marine mammals

OBJECTIVE: The objective of this study was to examine potential biochemical adaptations which may exist in marine mammals that would allow them to tolerate extreme pressure while diving to depth. Many investigators have examined the effects of hydrostatic pressure on individual enzymes which are part of complex metabolic pathways, particularly in marine teleosts (Somero, 1992). Very little has been done to examine similar questions in marine mammals, some of which dive to significant depths (Croll et al., 1992). Our goal was to examine the effect of hydrostatic pressure on glycolysis. We intended to take a multi-level approach to the problem, investigating the effect of hydrostatic pressure on lactate dehydrogenase kinetics as well as on whole cell glycolytic rate. Since glycolysis is a complex series of enzymatic reactions, knowing the effect of pressure on one enzyme in the path does not fully address the question of functional changes in the entire pathway. We were also hoping to assess the effect of hydrostatic pressure on Na^+,K^+-ATPase, a membrane-bound enzyme which, in humans, has been shown to be sensitive to hydrostatic pressure.

APPROACH: This project successfully examined two levels of potential biochemical adaptation. First, live red blood cells (RBCs) from marine and terrestrial mammals were subjected to 2 hours of pressure (2000 psi) at 37°C and their metabolic rates were analyzed. Second, tissue extracts of muscle were analyzed under pressure to determine changes in enzyme activity or affinity for substrate. Our attempts to study potential membrane-bound enzyme adaptations were unsuccessful because the techniques required to isolate membranes could not be conducted under field conditions, requiring us to use frozen blood cells for the preparation. The enzyme we were attempting to analyze (Na^+, K^+- ATPase) proved unstable when frozen *in situ*. This has prompted us to find an alternate method for isolating cell membranes which can be conducted under field conditions - preliminary tests are currently being undertaken.

ACCOMPLISHMENTS: We have been able to analyze metabolism of RBCs under pressure from 6 species of pinniped (including shallow and deep divers), *Tursiops spp.*, and 4 species of terrestrial mammals, including human. We have concluded that hydrostatic pressure does affect the rate of glycolysis (measured as lactate production) in RBCs of many species and that pinniped RBCs respond differently to pressure than do those of terrestrial mammals. Pinniped RBCs exhibited either very little change or an increase in glycolytic rate, while RBCs from 3 species of terrestrial mammals showed a marked decrease in glycolytic rate at pressure. Interestingly, the deepest diving seals (Weddell seals and northern elephant seals) showed the smallest effect of pressure while more shallow diving species (ringed seal and harbor seal) exhibited a more

dramatic response. The response of RBCs from shallow diving seals to pressure was opposite to the response of RBCs of terrestrial mammals.

We measured lactate produced/glucose consumed in RBCs of all species and found it to be significantly less than the theoretical value of 2 in all but *Tursiops spp.*, humans and elephant seal pups. This indicates that a significant amount of glucose is being used by an alternate pathway in most species. Shifts of glucose utilization rate and lactate/glucose at pressure suggest that the increase of glycolytic rate observed in RBCs of some pinniped species may be a result of a shift in metabolic pathway toward glycolysis. The decrease in glycolytic rate observed in RBCs of terrestrial mammals appears to be the result of a suppression of glucose utilization.

Red blood cells from humans and *Tursiops spp.* showed virtually no effect of hydrostatic pressure on glycolytic rate, rate of glucose utilization or lactate/glucose. In addition, lactate/glucose was 2, as theoretically predicted if all glucose consumed is being used in glycolysis. Castellini et al., 1992 have discussed the observation that glucose distribution in RBCs of odontocetes and primates is different than that of most mammals and may indicate a need to transport more glucose to the brain of these animals. This may also be reflected in different metabolism of glucose within the RBC.

Our studies indicate that hydrostatic pressure affects mammalian cardiac lactate dehydrogenase (LDH) kinetics. Unpressurized, maximum LDH activity (V_{max}) was higher in marine mammals for both substrates and cofactors. The K_m values for lactate and pyruvate were 36% higher for marine species than for terrestrial species. The K_m values for NAD^+ and NADH were similar between marine and terrestrial mammals. While there was no impact of 2000 psi on V_{max} or the K_m for pyruvate and NAD^+ in both marine and terrestrial mammals, pressure decreased lactate K_m by 23% and 21%, respectively and increased NADH K_m by 62% and 39%, respectively. Since K_m for lactate decreased and for NADH increased at 2000 psi, pressure may enhance the removal of lactate from cardiac tissues in marine and terrestrial mammals.

SIGNIFICANCE: While LDH was sensitive to hydrostatic pressure there was little difference between the marine and terrestrial mammals tested. Elevated pressure may enhance the removal of lactate from cardiac tissues of deeply diving mammals. The LDH kinetics results are not consistent with the observations made of whole cell glycolytic changes, in which marine and terrestrial mammals exhibited striking differences from each other. While tissue differences (cardiac vs RBC) may contribute to this disparity, it is suggestive that changes in LDH kinetics brought on by pressure may not allow one to predict overall flux changes in the glycolytic pathway. This is supported by observations of lactate/glucose and glucose utilization rates, which suggest that flux changes in glycolysis at pressure may be affected by shifts in metabolic pathways or suppression of glucose utilization, possibly by an effect on glucose transport. The results from examining the effect of pressure on whole cell glycolysis allow a context in which to examine regulation of individual enzymes or transporters.

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Somero, G.N. (1992). Adaptations to high hydrostatic pressure. *Annual Review of Physiology*, 54:557-577.

PRESENTATIONS AND ABSTRACTS:

Seminars:

Pressure, stress and cardiac function in marine mammals:
Scripps Institution of Oceanography, April 1994
University of Southern California medical school, April 1994
University of California, Santa Cruz, April 1994

Abstracts:

Castellini, M.A. and Castellini, J.M. (1993). Impact of pressure on RBC metabolism: Marine and terrestrial mammals. XXXII International Union of Physiological Sciences, Glasgow, Scotland.

Rivera, P.M. and M.A. Castellini. (1993). Lactate dehydrogenase activity in the heart muscle of diving marine mammals and terrestrial mammals. Tenth Biennial Conference on the Biology of Marine Mammals. Galveston, TX.

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Rivera, P.M. and Castellini, M.A. (1996). Cardiac lactate dehydrogenase activity in marine and terrestrial mammals: Response to pressure. The FASEB Journal, 10:A297. *Manuscript and Master's thesis in preparation.*

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