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Comparative Biochemistry and Physiology, Part A 147 (2007) 50–56
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Hemoglobin–oxygen-affinity and acid-base properties of blood from the fossorial mole-rat, *Cryptomys hottentotus pretoriae*

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Received 8 June 2006; received in revised form 7 November 2006; accepted 10 November 2006
Available online 28 November 2006

Abstract

Oxygen affinity and other hematological parameters in strictly subterranean mole-rats, *Cryptomys hottentotus* (subspecies *pretoriae)* were measured immediately upon capture and after 14–21 days in captivity. The pH, hematocrit, hemoglobin (Hb) concentration, blood oxygen content, 2,3 bisphosphoglycerate (2,3 BPG) concentration and oxygen dissociation curves (ODC), as well as tonometric measurements, were determined using whole blood. Additionally ODCs were also determined for stripped hemolysates of individual animals. Compared to other mammals, blood of freshly caught animals had low pH (7.32±0.22), elevated hematocrits (48.4±3.8 %) and significantly lower P_{50} values for whole blood (21.1±1.6 mm Hg at pH 7.4) than those reported for other similar-sized fossorial and terrestrial mammals. Blood carbon dioxide content (22.4±3.9 mMol L^{-1}), hemoglobin concentration (1.9±0.15 mMol L^{-1}), oxygen content (164.8±26 mL L^{-1}), bicarbonate concentrations (22.5±3.5 mMol L^{-1}) were within the range of values reported for similar-sized mammals. We conclude that high blood–oxygen affinity, low body temperature and possibly also high hematocrit enable *C. h. pretoriae* to maintain an adequate oxygen supply to the tissues in a potentially hypoxic burrow atmospheres, but that the blood of this species shows no exceptional CO_2 sensitivity or buffering capacity.

Keywords: Mole-rat; Whole blood; Hemoglobin; P_{50}; Acid-base balance; Hypercapnia

1. Introduction

Subterranean mammals have evolved a suite of structural and physiological features that enable them to exploit the ecological advantages afforded by an environment that is buffered from climatic extremes and largely protects against predation (Bennett et al., 1988; Buffenstein, 1996). These adaptations include independence of light in neuroendocrine control low resting metabolic rates coupled with low resting body temperature as well as morphological changes in the respiratory and cardiovascular systems (Widmer et al., 1997; Buffenstein, 2000).

The subterranean habitat is often characterized by both hypoxic and hypercapnic gaseous atmospheres (Ar, 1987; Arieli, 1990). Oxygen concentrations in burrows may regularly decrease to as low as 6–14%, with a concomitant rise in carbon dioxide concentrations to levels of 6–10% (Ar et al., 1977; Boggs et al., 1984; Roper et al., 2001). Such atypical gaseous conditions are attributed to respiratory exchanges of the resident animals, soil micro-organisms, plant roots and tubers, as well as to limited gas exchange with ambient air due to the physical properties of soils which affect permeability and the movement of gases (Lechner, 1976; Arieli, 1979). The degree of hypoxia and hypercapnia may be more severe in water-logged clay soils because gas diffusion rates are retarded owing to lower porosity and greater binding of gases to soil particle surfaces. The impact of hypoxic and hypercapnic conditions on soil inhabitants is most pronounced in animals that are continuously exposed to such unusual gaseous atmosphere, such as strictly subterranean mammals that seldom, if ever, leave the safe confines of a burrow system (Ar et al., 1977). Such animals may, when actively digging, experience potentially lethal levels of CO_2 and O_2 regardless of how well their burrows are ventilated (Lechner, 1976).

In response to hypoxia, air-breathing vertebrates may hyperventilate or increase the oxygen carrying capacity of blood. The

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1095-6433/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2006.11.016

oxygen affinity of blood depends upon the intrinsic affinity of hemoglobin for molecular oxygen, and the concentration of organic phosphates within red blood cells, which influences the interaction between hemoglobin and oxygen (Nikinmaa, 2001). Most studies examining effects of hypoxia have focused on animals living at high altitude (Samanja, 1997) and reported increased ventilation rates, elevated hematocrits as well as leftward shifts of oxygen dissociation curves indicating an enhanced oxygen affinity and improved oxygen exchange (Nikinmaa, 2001). Specific hematological adaptations to hypoxic/hypercapnic atmospheres in the burrows of subterranean mammals include raised hemoglobin content and high affinity to O_2, (Johansen et al., 1976; Arieli, 1979; Ar, 1987), elevated blood oxygen capacity (Lechner, 1976) and reduced sensitivity to CO_2 owing to better blood buffering capacity (Darden, 1972). Some of these physiological traits of subterranean mammals can be considered neotenous in that they resemble those of mammalian embryos (Ar, 1987).

Strictly subterranean Israeli blind mole-rats (genus *Spalax*) tolerate chronic hypoxia and hypercapnia far better than laboratory rats, and possess physiological adaptations of the respiratory and circulatory systems that underlie this tolerance (Ar et al., 1977; Widmer et al., 1997). When maintained under hypoxic and hypercapnic conditions, blood of these rodents contains more CO_2 at any given pH than the blood of most mammals.

Both the properties of the oxygen dissociation curve and ability of organic phosphates to reversibly alter hemoglobin–oxygen affinity are affected by hypoxia and/or hypercania (Jelkmann et al., 1981). There is a considerable body of evidence to show that the high oxygen–hemoglobin affinity of burrowing animals is genetically determined (Nikinmaa, 2001). Some mole-rats, for instance, manufacture hemoglobin with a high oxygen affinity (Johansen et al., 1976; Kleinschmidt et al., 1985) and respond to short term changes in the degree of hypoxia/hypercapnia by modifying the number of red blood cells and altering the concentrations of 2,3 BPG, in addition to attenuating the interaction between 2,3 BPG, hemoglobin and pH (Johansen et al., 1976; Boggs, 1995).

The Highveld mole-rat, *Cryptomys h. pretoriae*, is a social bathyergid found in a wide spectrum of southern African subterrestrial habitats, ranging from highly porous sandy soils to water-logged clays. Like other fossorial rodents, it has a low resting metabolic rate with an average body temperature of 34.4 °C (Bennett et al., 1992), which reduces oxygen requirements and is presumably adaptive under hypoxic conditions. Broekman et al. (2006) recently examined hematological parameters and oxygen equilibrium curves in two populations of the Lesotho mole-rat (*C. h. mahali*), and concluded that while these mammals are hypoxia-adapted, oxygen-transporting properties did not change markedly with increased altitude. However, their analyses were based on thawed whole blood samples in which the freezing process lysed the red cells. Nothing is known of the respiratory physiology of fresh whole blood samples and hemoglobin solutions, and buffering capacity when exposed to large loads of CO_2, thus prompting this study. Since gas diffusion rates depend mainly on soil porosity, we targeted colonies inhabiting heavy clay soils within which hypoxic/hypercapnic conditions are most likely to prevail. We also assessed if animals acclimated to laboratory atmospheres exhibit a similar response to freshly caught animals, to establish if these characteristics reflect irreversible properties or changeable responses to existing environmental conditions.

2. Materials and methods

2.1. Animal capture and handling

Specimens of *C. h. pretoriae* (mean body mass: 115 g, SD: 35.0 g) were captured live in late summer (February–March) using tunnel traps (modified from Hickman, 1979) baited with sweet potato. Traps were set on the riverbanks of the Loopspruit River near Potchefstroom (S 26. 43.111: E 27. 08.204), where soils are eutrophic with a plinthic catena and a high clay content (De Villiers and Mangold, 2002). Upon capture, animals were placed in 20 l buckets containing 15–20 cm of soil and transferred to the laboratory for measurements of hematological parameters within 3 h. A second group of mole-rats was caught in similar soils on the riverbank of the Mooi River (S 26 40.599: E 027 06.002). These animals were subjected to measurements within 3 h of capture, and again 14–21 days later. After initial blood sampling, animals were housed individually in large (1.5 m × 1.2 m × 1.4 m) unsealed containers (containing approximately 15 cm of moist soil taken from the capture sites) in a darkened room at 20–25 °C and supplied with sweet potatoes *ad libitum*. After the second blood sample was drawn mole-rats were released at the capture sites.

2.2. Blood sampling

Immediately prior to sampling the mole-rats were warmed for 20–30 min in a ventilated laboratory oven at 30 °C to stimulate vasodilation. Blood samples (300 μL) were obtained by puncturing the brachial vein or other obvious blood vessels on the ventral surface of the hind feet pad using a 21G sterile hypodermic needle, collected in heparinized capillary tubes, and kept on ice for 1–2 h before analysis.

2.3. Hematology measurements

Blood pH was measured (to three decimal places) immediately at 35 °C (the normal body temperature of this species) using the "pistol" type micro-electrode unit, E5021 and capillary electrode (G297/G2, Radiometer) with a PHM 73 blood gas monitor (Radiometer, Copenhagen) using pH calibration standards in sealed capsules from Radiometer. Samples were then centrifuged at 3000 *g* in micro-hematocrit tubes for 5 min to determine hematocrit. Hemoglobin concentration, expressed both as gram percent and moles per liter, was determined spectrophotometrically and calculated from the millimolar extinction coefficient (14.37 at 542 nm) for human hemoglobin using a molecular mass of 68 000 (Johansen et al., 1976).

The oxygen content of freshly collected blood was determined using a 500 μL Tucker-cell (TC 500, Strathkelvin