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Purine nucleoside phosphorylase and xanthine oxidase activities in erythrocytes and plasma from marine, semiaquatic and terrestrial mammals

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ABSTRACT

Purine nucleoside phosphorylase (PNP) and xanthine oxidase (XO) are key enzymes involved in the purine salvage pathway. PNP metabolizes purine bases to synthetize purine nucleotides whereas XO catalyzes the oxidation of purines to uric acid. In humans, PNP activity is reported to be high in erythrocytes and XO activity to be low in plasma; however, XO activity increases after ischemic events. XO activity in plasma of northern elephant seals has been reported during prolonged fasting and rest and voluntary associated apneas. The objective of this study was to analyze circulating PNP and XO activities in marine mammals adapted to tolerate repeated cycles of ischemia/reperfusion associated with diving (bottlenose dolphin, northern elephant seal) in comparison with semiaquatic (river otter) and terrestrial mammals (human, pig). PNP activities in plasma and erythrocytes, as well as XO activity in plasma, from all species were quantified by spectrophotometry. No clear relationship in circulating PNP or XO activity could be established between marine, semiaquatic and terrestrial mammals. Erythrocytes from bottlenose dolphins and humans are highly permeable to nucleosides and glucose, intraerythrocyte PNP activity may be related to a release of purine nucleotides from the liver. High-energy costs will probably mean a higher ATP degradation rate in river otters, as compared to northern elephant seals or dolphins. Lower erythrocyte PNP activity and elevated plasma XO activity in northern elephant seal could be associated with fasting and/or sleep- and dive-associated apneas.

1. Introduction

Breath-hold (apnea) diving is part of the natural history of marine mammals, including whales and seals. Terrestrial mammals, however, are susceptible to drastic changes in oxygen levels (Noren et al., 2001; Ramirez et al., 2007; Zenteno-Savín et al., 2012). Among marine mammals, breath-holding capacity and diving behavior are species specific. Bottlenose dolphins (*Tursiops truncatus*) usually dive from 0.2 to 10 min and to depths between 4 and 700 m (Lockyer and Morris, 1987; Klatsky et al., 2007). Northern elephant seals can perform routine dives to depths of 400 m lasting for 20–25 min (Le Boeuf et al., 1988, 1993, 2000; Stewart and DeLong, 1995; Stewart, 1997; Davis et al., 2001). Semiaquatic mammals, such as river otters (*Lontra longicaudis annectens*), are also capable of breath-hold diving; however, they usually dive close to the surface and for very short periods of time (4 min; Yeates et al., 2007). Humans lose consciousness if the brain is deprived of oxygen during 15–20 s and suffocate if ventilation is interrupted during 3–4 min (Ramirez et al., 2007).

Breath-hold diving is characterized by reduced blood flow (ischemia) and decreased tissue oxygen content (hypoxia). During a dive, blood flow to the brain and heart in seals may decrease 50 and 90%, respectively, from pre-dive levels; in kidney, liver and intestines, perfusion appears to decrease completely (Elsner, 1999; Folkow and Blix, 2010). The response to ischemia/reperfusion and hypoxia/reoxygenation includes low heart rate (bradycardia), and peripheral vasoconstriction

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(Kooyman and Ponganis, 1998; Elsner, 1999). Diving performance depends on oxygen storage and utilization, the concentration of myoglobin (Mb) and hemoglobin (Hb), the extent of organ perfusion, locomotor behavior (stroke frequency), the degree of ischemia and the aerobic dive limit (ADL) of each species (Kooyman and Ponganis, 1998; Ponganis et al., 2011). For example, emperor penguins (*Aptenodytes forsteri*) can dive to >100 m deep for 20 min (Sato et al., 2011) and are able to optimize oxygen consumption, Mb desaturation rates, and the extent of muscle perfusion according the type of dive (Williams et al., 2011). Northern elephant seals adjust blood flow, blood oxygen reduction, bradycardia and Hb saturation according to the degree of ischemia; during routine dives, the level of these physiological responses is between those observed in forced submersions and sleep apnea (Meir et al., 2009). Despite physiological adjustments during diving, depletion and degradation of ATP stores lead to accumulation of purine nucleotides (Elsner et al., 1998; Elsner, 1999; Ponganis et al., 2008). The enzymes purine nucleoside phosphorylase (PNP) and xanthine oxidase (XO) have a crucial role in the metabolism of adenosine during adenosine triphosphate (ATP) degradation (Åberg et al., 2010). PNP catalyzes the reversible phosphorolysis of purine nucleosides, inosine to hypoxanthine (HX) and guanosine to guanine (Walker et al., 2011). XO catalyzes the oxidation of HX to xanthine and further to uric acid (Muxfeldt and Schaper, 1987); both enzymes are involved in the purine salvage pathway.

The kidney and heart of a non-diving mammal (*Sus scrofa*) accumulate higher levels of HX than those of a diving mammal (*Phoca hispida*) during *in vitro* ischemia (Elsner et al., 1998). In humans, the highest PNP activities are reported in the kidney, peripheral lymphocytes, erythrocytes and granulocytes (Bzowska et al., 2000). PNP activities have been quantified in myocardium of humans, dogs, pigs, rabbits, guinea pigs and rats, particularly in pericytes, vascular endothelium and nerve cells (Borgers and Thone, 1992). XO activity is found in several tissues in humans; the higher activity has been reported in liver and jejunum (Kooij et al., 1992). However, low or no XO activity has been reported in pig, rabbit and human heart (Muxfeldt and Schaper, 1987). XO activity has been reported in the circulation of humans, rabbits and northern elephant seals (*Mirounga angustirostris*) (White et al., 1996; Newaz and Adeeb, 1998; Vázquez-Medina et al., 2011). Increased plasma XO activity in humans has been related to liver diseases, myocardial infarction, and/or ischemia/reperfusion events (Friedl et al., 1990; Yokoyama et al., 1990; de Jong et al., 2000; Zhao et al., 2008). The goal of this study was to analyze PNP activity in erythrocytes and plasma, as well as XO activity in plasma, from marine and semiaquatic mammals (bottlenose dolphin, northern elephant seal, river otter) with different diving capacities (i.e. diving depth and duration), and to compare them with those in terrestrial, non-diving mammals (human, pig).

2. Materials and methods

2.1. Subjects

All samples were collected under the corresponding permissions, valid at the time of sampling. Volunteer human subjects were informed of the procedure and goals of the project, and were requested to give their written consent prior to sample collection. Sampling and experimental procedures were examined and authorized by the hospital's ethics committee and by the Capítulo Baja California Sur de la Academia Nacional Mexicana de Bioética, A.C.

All samples were collected from healthy individuals. No pregnant or lactating organisms were included in this study. Overnight fasting was a prerequisite for all subjects, except northern elephant seals, which were sampled during the natural fast they undergo when molting (estimated fasting duration at sampling time, 2–3 weeks). Sex and morphometric data for sampled individuals are summarized in Table 1. The sex and weight of pigs were not determined.

2.2. Sample collection

Peripheral blood samples were taken from humans (*Homo sapiens*, n = 11), domestic pigs (*Sus scrofa*, n = 11), bottlenose dolphins (*Tursiops truncatus*, n = 11), northern elephant seals (*Mirounga angustirostris*, n = 11) and river otters (*Lontra longicaudis annectens*, n = 4). Blood samples were collected from bottlenose dolphins under human care at Cabo Dolphins, Los Cabos, Baja California Sur, Mexico; these dolphins are part of a preventive medical program and are trained for routine veterinary check-ups (including blood drawing), thus, distress during the procedure is minimized. Blood was drawn from the tail fluke using a 21 gauge (G) butterfly needle BD™. Blood from northern elephant seals was collected from sedated animals at Año Nuevo State Reserve, CA, USA, as previously reported (Champagne et al., 2005; Vázquez-Medina et al., 2011). Blood samples from river otters were obtained from animals held at Acuario de Veracruz, Mexico. Otters were anesthetized using a mixture of xylazine (1.5 mg kg^{-1}) and ketamine (15 mg kg^{-1}) and blood was drawn from the cephalic vein in the right forelimb, using a 21 G × 3/4″ × 7″ needle and BD Vacutainer® Safety-Lok blood collection set. Human blood samples were collected from healthy volunteers at a local hospital; samples were drawn by venipuncture from the cubital fossa veins, following routine procedures. Domestic pigs were sampled at the local slaughterhouse, blood samples were collected directly from the jugular vein immediately after animals were sacrificed. All blood samples were collected under aseptic conditions in Vacutainer® tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. To recover the plasma, samples were centrifuged on site in a field centrifuge (Mobilespin, Grandview, MO, USA) at 850 × *g* for 10 min. Buffy coat was discarded, red blood cells were washed using cold saline solution (0.9%). All samples were kept on ice during transportation, frozen by immersion in liquid nitrogen, and stored at −80 °C until analyzed. Intraerythrocyte content was obtained by osmotic shock with cold distilled water; to ensure complete hemolysis, samples were frozen and thawed twice followed by centrifugation at 9000 × *g* for 15 min.

2.3. Biochemical analyses

2.3.1. Purine nucleoside phosphorylase (EC 2.4.2.1) activity

PNP activity was quantified in plasma and intraerythrocyte content by using a colorimetric assay as described by Chu et al. (1989). Briefly, each sample was incubated with potassium phosphate buffer (22 mM, pH 7.5) containing XO (167 U), horseradish peroxidase (2000 U), 4-aminoantipyrine (160 mM), potassium ferrocyanide (120 μM), 3,5 dichloro-2-hydroxybenzenesulfonic acid (8 mM), and inosine (12 mM). Production of N-(4-antipyryl)-3-chloro-5-sulfonate-p-benzoquinone-monoimine was followed at 520 nm in a spectrophotometer (Beckman Coulter DU 800, Fullerton, CA, USA) and the change in absorbance was recorded every 5 s for 180 s. Bilirubin interference was reduced by addition of ferrocyanide. Results are expressed as units (U) mg^{-1} protein. One unit of PNP activity is defined as the amount of enzyme necessary to deplete 1 μM of inosine per min at 25°C.

Table 1
Sex and morphometric data from the marine, semiaquatic and terrestrial mammal species included in this study.

	Sex n males/n females	Age (years)	Weight (kg)
Human	4/7	33.1 ± 4.9	62.6 ± 11.4
Pig	NA	1.5 ± 0.7	NA
Bottlenose dolphin	5/6	9.3 ± 3.9	190.8 ± 31.5
Northern elephant seal	4/7	~0.8	126.3 ± 13.5
River otter	2/2	3.3 ± 1.4	6.84 ± 0.8

NA = data not available. Data are presented as mean ± standard deviation.