Comparative Biochemistry and Physiology, Part A 206 (2017) 11–16

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Na^+/K^+-ATPase activity in the anoxic turtle (*Trachemys scripta*) brain at different acclimation temperature

Jonathan A.W. Stecyk [a,b,*], Anthony P. Farrell [b,c], Matti Vornanen [d]

[a] Department of Biological Sciences, University of Alaska Anchorage, Anchorage, AK 99508, USA
[b] Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada
[c] Faculty of Land and Food Systems, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada
[d] University of Eastern Finland, Department of Environmental and Biological Sciences, P.O. Box 111, 80101 Joensuu, Finland

ARTICLE INFO

Article history:
Received 3 October 2016
Received in revised form 23 December 2016
Accepted 4 January 2017
Available online 13 January 2017

Keywords:
[^3H]ouabain binding
Anoxia
Channel-arrest
Sodium pump
Hibernation

ABSTRACT

Survival of prolonged anoxia requires a balance between cellular ATP demand and anaerobic ATP supply from glycolysis, especially in critical tissues such as the brain. To add insight into the ATP demand of the brain of the anoxia-tolerant red-eared slider turtle (*Trachemys scripta*) during prolonged periods of anoxic submergence, we quantified and compared the number of Na^+-K^+-ATPase units and their molecular activity in brain tissue from turtles acclimated to either 21 °C or 5 °C and exposed to either normoxia or anoxia (6 h 21 °C; 14 days at 5 °C). Na^+-K^+-ATPase activity and density per g tissue were similar at 21 °C and 5 °C in normoxic turtles. Likewise, anoxia exposure at 21 °C did not induce any change in Na^+-K^+-ATPase activity or density. In contrast, prolonged anoxia at 5 °C significantly reduced Na^+-K^+-ATPase activity by 55%, which was largely driven by a 50% reduction of the number of Na^+-K^+-ATPase units without a change in the activity of existing Na^+-K^+-ATPase pumps or α-subunit composition. These findings are consistent with the "channel arrest" hypothesis to reduce turtle brain Na^+-K^+-ATPase activity during prolonged, but not short-term anoxia, a change that likely helps them overwinter under low temperature, anoxic conditions.

1. Introduction

The primary consumer of metabolic energy (i.e., adenosine triphosphate; ATP) in the vertebrate brain is the Na^+-K^+-ATPase, an electrogenic, transmembrane ATPase (Erecinska et al., 2004). This ion pump accounts for 50–80% of neuronal energy consumption due to its role in establishing the Na^+ and K^+ gradients that are critical for neuronal processes and functions, including the regulation of membrane potential, electrical excitability, neurotransmitter release and uptake and osmotic balance. The continued functioning of brain Na^+-K^+-ATPase is supported by the efficient generation of ATP by the oxidative metabolism of glucose and lactate (Falkowska et al., 2015). Consequently, the brain of most vertebrate species, including mammals, is notoriously sensitive to oxygen deprivation. Under conditions of severe hypoxia, anoxia and/or ischemia, the intense energy demand of neurons rapidly outstrips anaerobic ATP production, leading to a pronounced fall of cellular ATP concentration (Boutilier, 2001). Subsequently, within minutes, Na^+-K^+-ATPase activity decreases, ionic gradients are lost and a catastrophic sequence of events is initiated that culminates in irreversible neuronal damage (Lipton, 1999).

By contrast, the brain of the red-eared slider turtle (*Trachemys scripta*) can endure 24 h of anoxia at 20–25 °C (Jackson, 2000). This extraordinary ability stems from a multitude of constitutive, as well as induced, physiological mechanisms that permit a balance between brain ATP supply and demand (reviewed by Bickler and Buck, 2007; López-Barneo et al., 2010; Lutz and Milton, 2004; Milton and Prentice, 2007; Nilsson, 2001). Overall, a strong and coordinated suppression of metabolic and electrical activity reduces ATP demand to a level that can be supported by the limited ATP generated from anaerobic glycolysis (Lutz and Nilsson, 2004). Central to the energy conservation strategy is a reduction of Na^+-K^+-ATPase activity. Whole animal metabolic rate during anoxia at 20–24 °C is 15–18% of that normoxic warm-acclimated turtles (Herbert and Jackson, 1985b; Jackson, 1968) and turtles exposed to 24 h of anoxia at 20 °C exhibited a 30% reduction of Na^+-K^+-ATPase activity in all major parts of the brain, which was subsequently reversed upon reoxygenation (Hylland et al., 1997). Thus, unlike in the brain of anoxia-sensitive vertebrates, the fall of Na^+-K^+-ATPase activity in the turtle brain during anoxia at high temperature is compensatory and adaptive, rather than pathological as in mammals.

Although the ability of *T. scripta* to recover from a 24-h period of anoxia at high temperatures is impressive, its anoxia tolerance is even more striking at the low temperature (3–5 °C) of the ice-covered ponds in which the turtle overwinters. At these temperatures, the turtle can survive for many weeks without oxygen (Ultsch, 1985; Warren et

* Corresponding author at: Department of Biological Sciences, University of Alaska Anchorage, 3211 Providence Drive, Anchorage, AK 99508, USA.
E-mail address: jstecyk@alaska.edu (J.A.W. Stecyk).

http://dx.doi.org/10.1016/j.cbpa.2017.01.002
1095-6433/

al., 2006). This prolonged cold-temperature anoxia survival is possible due to profound temperature- and anoxia-induced reductions in metabolic rate that collectively, serve to minimize ATP consumption to a level that can be supported by anaerobic metabolism alone (Herbert and Jackson, 1985b; Jackson, 2000). Firstly, with acclimation to low temperature, turtles exhibit inverse thermal compensation. Physiological processes, especially energy consuming ones, are actively down-regulated, beyond decreasing passively with cold temperature such that they are reset to benefit the survival of winter anoxia. In particular, whole animal metabolic rate is reduced by 94% (temperature coefficient (Q_{10}) = 5.3) with acclimation from 20 °C to 3 °C (Herbert and Jackson, 1985b), and concurrently, systemic cardiac power output, which reflects cardiac ATP demand, is reduced by 5- to 15-fold (Q10 = 4.7–8.8, Hicks and Farrell, 2000; Stecyk et al., 2004). With subsequent anoxia exposure metabolic rate is further reduced. Whole animal metabolic rate of cold, anoxic turtles is <10% of the cold, normoxic rate (Herbert and Jackson, 1985b) and systemic cardiac power output of 5 °C-acclimated turtles is 7- to 20-fold less in anoxia than in normoxia at 5 °C (Hicks and Farrell, 2000; Stecyk et al., 2004).

While studies on whole animal and cardiac metabolic rate have factored in the effect of decreased temperature, most previous investigations of the cellular anoxia tolerance of the turtle brain have not. As a result, to the best of our knowledge, it is not known how turtle brain Na^+-K^+-ATPase activity is affected by either acclimation to low temperature or by prolonged anoxia exposure at low temperature. Therefore, the aim of the present study was to characterize Na^+-K^+-ATPase activity in brain of *T. scripta* and compare the effects of anoxic submergence at high temperature (6 h at 21 °C) with those of acclimation to low temperature (5 °C) and prolonged anoxia exposure (14 days) at 5 °C. Because a reduction of brain Na^+-K^+-ATPase activity to facilitate the conservation of metabolic energy could theoretically be brought about by a reduction in the density of Na^+-K^+-ATPase pumps per unit of tissue or a reduction of the activity of existing Na^+-K^+-ATPase pumps, we quantified both the number of Na^+-K^+-ATPase units and their molecular activity. We hypothesized that cold acclimation, and to a greater extent, prolonged anoxia at 5 °C, would induce a marked reduction of Na^+-K^+-ATPase units and activity in-line with the "channel arrest" hypothesis. The hypothesis predicts that the long-term survival of hypothermic and/or anoxic conditions is facilitated by the coordinated suppression of transmembrane ion flow through ion channels and active ion transport by ion pumps such as the Na^+-K^+-ATPase (Hochachka, 1986; Lutz et al., 1985).

2. Materials and methods

2.1. Experimental animals and ethical approval

Thirty-six red-eared sliders (*Trachemys scripta*, Gray) of both sexes and with body masses ranging between 124 and 344 g (238 ± 63.5 g, mean ± SD) were obtained from a commercial supplier (Lemberger Inc., Oshkosh, WI, USA). The exposure design for the turtles was normoxia and anoxia exposure × 21 °C– and 5 °C– acclimation. Turtles studied at 21 °C were held indoors in aquaria under a 12 h:12 h L:D photoperiod, had free access to basking platforms and water for diving, and were fed several times a week with commercial turtle food pellets. The turtles studied at 5 °C were kept in aquaria with shallow water (3–4 cm) under a 12 h:12 h L:D photoperiod in a temperature-controlled room set to 5 °C. The turtles were acutely exposed to 5 °C, but the exposure duration was for a minimum of 5 weeks prior to tissue sampling or anoxia exposure to allow adequate time for cold-acclimation (Hicks and Farrell, 2000). In addition, acclimation to 5 °C occurred during winter months, and turtles were fasted during the acclimation period. Normoxic turtles were sampled from these conditions. For prolonged anoxia, 21 °C turtles were exposed to anoxia for 6 h and 5 °C turtles for 14 days. The anoxic conditions were achieved by placing individual turtles into an enclosed, water-containing plastic chamber that still allowed access to air for 24 h, after which the plastic chamber was filled with water, continuously bubbled with N_2 and access to the water surface denied by means of mesh suspended below the surface of the water. Water P_{O2} was monitored intermittently throughout anoxia exposure with a WTW Multiline P4 meter with CellOx 325 electrode (Wissen-schaftlich-Technische Werkstätten, Weilheim, Germany). The detection limit of 0.1 mg l^{-1} (=0.16 kPa) used as the limit for anoxia. 21 °C-acclimated turtles were not comatose following the anoxia exposure, whereas 5 °C-acclimated turtles were found to be unresponsive to tactile stimulation. All procedures were performed with the consent of the local committee for animal experimentation (permission No. 21102004).

2.2. Preparation of brain homogenates

Turtles were killed by decapitation. For anoxia-exposed animals, decapitation occurred underwater such that the animals did not have the possibility to breath air. Within minutes of the initiation of animal handling, the whole brain was dissected, blotted dry, and weighed to the nearest 0.1 mg and immediately frozen at −40 °C. Within three months, dilute brain homogenates (5%) were prepared by homogenizing individual brains in 20 volumes of ice-cold Tris•HCl (50 mM, pH 7.4 at 20 °C) buffer using a Teflon-glass homogenizer and four 20 s bursts at 2200 rpm. The homogenate was divided into equal portions and used for the quantification of Na^+-K^+-ATPase activity, the number of Na^+-K^+-ATPase units and the affinity of the homogenates for ouabain.

2.3. Determination of Na^+-K^+-ATPase activity

Na^+-K^+-ATPase activity was determined from the release of inorganic phosphate (Atkinson et al., 1973), as previously described in detail (Vornanen and Paajanen, 2006). Briefly, maximal Na^+-K^+-ATPase activity was obtained as the difference in inorganic phosphate liberated in the presence and absence of 3 mM ouabain in a final volume of 1.0 ml of the ATPase medium that contained (in mM) 5 Na_2ATP, 5 $MgCl_2$, 20 KCl, 100 NaCl, 50 Tris•HCl, 1 EGTA, and 5 NaN_3, at pH 7.2. After a 10 min pre-incubation of the sample in the medium, the reaction was initiated with the addition of 25 μl of 100 mM ATP stock solution. An incubation period of 30 min at 25 °C was utilized for all samples because Na^+-K^+-ATPase activity of crude brain homogenates cannot be reliably measured at low temperature (Vornanen and Paajanen, 2006). Under these assay conditions, the release of inorganic phosphate was linear with time up to the end point of the incubation.

2.4. Quantification of Na^+-K^+-ATPase units

The number of Na^+-K^+-ATPase pumps was determined by high-affinity binding of [^3H]ouabain (Amersham, Little Chalfont, UK) to the alpha subunit of the Na^+-K^+-ATPase following procedures previously described in detail (Vornanen and Paajanen, 2006). Briefly, [^3H]ouabain binding was performed by incubating 0.5 ml of solution (4 mM H_3PO_4, 4 mM $MgCl_2$, 50 mM Tris•HCl, pH 7.4)(Matsui and Schwartz, 1968) that contained 0.5 mg of wet tissue with 4 nM [^3H]ouabain with for 3 h at 20 °C. The reaction was terminated with the addition of 6 ml of ice-cold wash buffer (4 mM $MgCl_2$, 50 mM Tris•HCl, pH 7.4 at 20 °C) and immediately filtered through a Whatman GF/B filter (Merck, Poole, UK) with three 6 ml washes of cold buffer. The filter was then soaked in 10 ml of scintillant (Ready Protein+, Beckman), and the amount of [^3H]ouabain bound to the filter was quantified by liquid scintillation counting (Wallac 1414 WinSpectral, Wallac, Finland). Unspecific binding in the presence of 100 μM unlabeled ouabain (Sigma, Poole, UK) was subtracted from the total binding to obtain specific binding.