Comparative Biochemistry and Physiology, Part A 184 (2015) 34–40

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Adaptation of oxidative phosphorylation to photoperiod-induced seasonal metabolic states in migratory songbirds

Amit Kumar Trivedi [a], Shalie Malik [b], Sangeeta Rani [b], Vinod Kumar [a,*]

[a] DST-IRHPA Center for Excellence in Biological Rhythm Research, IndoUS Center for Biological Timing, Department of Zoology, University of Delhi, Delhi 110007, India
[b] DST-IRHPA Center for Excellence in Biological Rhythm Research, Department of Zoology, University of Lucknow, Lucknow 226 007, India

ARTICLE INFO

Article history:
Received 12 November 2014
Received in revised form 16 January 2015
Accepted 19 January 2015
Available online 28 January 2015

Keywords:
Bunting
Energy generation
Migration
Oxidative phosphorylation
TCA cycle

ABSTRACT

Eukaryotic cells produce chemical energy in the form of ATP by oxidative phosphorylation of metabolic fuels via a series of enzyme mediated biochemical reactions. We propose that the rates of these reactions are altered, as per energy needs of the seasonal metabolic states in avian migrants. To investigate this, blackheaded buntings were photoperiodically induced with non-migratory, premigratory, migratory and post-migratory phenotypes. High plasma levels of free fatty acids, citrate (an intermediate that begins the TCA cycle) and malate dehydrogenase (mdh, an enzyme involved at the end of the TCA cycle) confirmed increased availability of metabolic reserves and substrates to the TCA cycle during the premigratory and migratory states, respectively. Further, daily expression pattern of genes coding for enzymes involved in the oxidative decarboxylation of pyruvate to acetyl-CoA (*pdc* and *pdk*) and oxidative phosphorylation in the TCA cycle (*cs*, *odgh*, *sdhd* and *mdh*) was monitored in the hypothalamus and liver. Reciprocal relationship between *pdc* and *pdk* expressions conformed with the altered requirements of acetyl-CoA for the TCA cycle in different metabolic states. Except for *pdk*, all genes had a daily expression pattern, with high mRNA expression during the day in the premigratory/migratory phenotypes, and at night (*cs*, *odhg*, *sdhd* and *mdh*) in the nonmigratory phenotype. Differences in mRNA expression patterns of *pdc*, *sdhd* and *mdh*, but not of *pdk*, *cs* and *odgh*, between the hypothalamus and liver indicated a tissue dependent metabolism in buntings. These results suggest the adaptation of oxidative phosphorylation pathway(s) at gene levels to the seasonal alternations in metabolism in migratory songbirds.

1. Introduction

Organisms maintain energy balance by equalizing energy (food) intake and energy expenditure, irrespective of the body size. Energy consumed in excess of its expenditure is converted into liver glycogen, muscle protein and adipose tissue triglycerides. These metabolic fuels are utilized by cellular respiration during the periods of starvation. All eukaryotic cells under aerobic conditions produce chemical energy in the form of ATP by oxidative phosphorylation of metabolic fuels, via a series of enzyme mediated biochemical reactions in the tricarboxylic acid (TCA) cycle (Krebs, 1937).

The acetyl coenzymeA (acetyl-CoA) produced from pyruvate, the end product of glycolysis and β-oxidation of sugars and fatty acids, respectively (Fell and Small, 1986; Suagee et al., 2010), begins the oxidative phosphorylation. The mitochondrial pyruvate dehydrogenase complex (pdc) connects the glycolytic and TCA cycles (Harris et al., 2002; Patel and Korotchkina, 2006). It catalyses the oxidative decarboxylation of pyruvate to the acetyl-coA, as controlled by the pyruvate dehydrogenase kinase (pdk) activity. The pdk phosphorylates pdc and hence by reducing the pdc activity lowers acetyl CoA supply to the TCA cycle. The entry of acetyl-CoA in the TCA cycle is a critical step in driving the oxidative phosphorylation via a number of intermediates, which beginning with the oxaloacetate includes α-ketoglutarate, succinate and malate. These substrates mediated by citrate synthase (cs), α-ketoglutarate dehydrogenase (odgh), succinate dehydrogenase (sdhd) and malate dehydrogenase (mdh) enzymes form the citrate, succinyl-CoA, fumarate and oxaloacetate, respectively. Hence, the level of substrates and/or enzymes may be taken as indices of the reaction rates of the TCA cycle.

Migratory birds adopt a metabolic strategy that helps them meet the energy costs of twice-a-year travel in between their breeding and wintering grounds, which in many species (long distance migrants) may be separated by several thousands of kilometers (Ramenofsky, 2011). These birds show daily and seasonal transitions in their physiology and behavior with the onset and end of the migratory seasons (Ramenofsky et al., 1999). In particular, they show hyperphagia, fatten and gain weight, and accumulate carbohydrates and lipids as liver glycogen and adipose tissue triglycerides, respectively, in the days prior to migration (Jain and Kumar, 1995; Jenni and Jenni-Eiermann, 1998; Ramenofsky et al., 1999; McWilliams et al., 2004; Weber, 2009; Singh, 2013; Trivedi et al., 2014). These metabolic

* Corresponding author.
E-mail addresses: drvkumar11@yahoo.com, vkumar@zoology.du.ac.in (V. Kumar).

http://dx.doi.org/10.1016/j.cbpa.2015.01.011
1095-6433/

reserves are catabolized to provide inflight fuels during migration. Inflight fuelling is almost exclusively met by the oxidation of fatty acids supplied by adipose tissue via circulation (Jenni and Jenni-Eiermann, 1998; McWilliams et al., 2004; Weber, 2009). Hence, changes in the plasma levels of free fatty acid may serve as the best indicator of the seasonal metabolic states (Landys et al., 2005).

Here, we propose that the mRNA expressions of genes coding for enzymes that mediate biochemical reactions in the TCA cycle pathway show significant differences between seasonal metabolic states in migratory songbirds. To investigate this, migratory blackheaded buntings were photoperiodically induced with non-migratory, premigratory, migratory and post-migratory phenotypes, with each phenotype considered as a life history state, LHS (Wingfield, 2008). First, we measured the plasma levels of free fatty acid and citrate (an intermediate substrate that begins the TCA cycle) and mdh enzyme (involved at the closing end of the TCA cycle) to test changes in the availability of metabolic reserve and substrates in the TCA cycle, respectively. Then, the daily expression of genes coding for enzymes involved in the oxidative decarboxylation of pyruvate to acetyl-CoA (*pdc* and *pdk*) and oxidative phosphorylation in the TCA cycle (*cs*, *odgh*, *sdhd* and *mdh*) was measured in the hypothalamus and liver. In buntings, the hypothalamus is the regulatory center for seasonal migratory and reproductive responses and shown to contain the putative seasonal clock (Rastogi et al., 2011, 2013), while the liver is known as the principal metabolic center (Singh, 2013). The prediction was that low and high *pdc* and *pdk* expression levels would correspond to the low and high acetyl-CoA requirements in the TCA cycle in the non-migratory and migratory states, respectively. Similar metabolic state dependence of mRNA expression was predicted for genes coding for the TCA cycle enzymes. We expected a day–night difference in mRNA expression levels because of the parallel shift in activity behavior and neuronal activity from predominantly day active to the predominantly night active when blackheaded buntings switch from non-migratory to the migratory state (Rastogi et al., 2011, 2013). Differences in gene expression pattern were also expected between the hypothalamus and liver, since the liver changes in all probability would account for the metabolism of metabolic reserves in buntings, especially during the migration linked metabolic states.

2. Material and methods

2.1. Animals and maintenance

Blackheaded bunting (*Emberiza melanocephala*) is a long distance night-migratory Palearctic–Indian songbird that overwinters in India (~25°N). It exhibits seasonal phenotypes linked with migration (fattening and weight gain, and *Zugunruhe* — the nighttime migratory restlessness in caged birds during migratory season) (Gwinner and Czeschlik, 1978) and reproduction (gonadal maturation) on exposure to long days, and remains unstimulated under short days with sensitivity maintained to long day stimulation (Jain and Kumar, 1995; Misra et al., 2004; Rani et al., 2006; Rastogi et al., 2011). Adult male buntings procured from overwintering flocks in late February were acclimated to the captive conditions for a week under natural day length and temperature conditions (NDL). Thereafter, they were brought indoors and maintained on short days (8 h light:16 h darkness, 8 L:16D) for about 24 weeks, until the beginning of the experiment (= photosensitive birds). At this time, they had no fat deposition, weighed about 25 g, were day active (i.e. no *Zugunruhe*) and had reproductively immature testes (testis volume, TV = ~0.52 mm^3). The temperature was maintained at 22 ± 2 °C, and food was provided ad libitum. The study was carried out at the Department of Zoology, University of Lucknow, Lucknow, India, in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC).

2.2. Experiment

Photosensitive birds (n = 46) were distributed in four groups (groups 1–4; n = 10–12 birds per group) and singly housed in cages (size = 60 × 40 × 35 cm) placed in the photoperiodic boxes (size = 71 × 67 × 48 cm) providing 8 L:16D (L = 100 lx; D = <1 lx). After a week, groups 2–4 birds were subjected to long days (13 L:11D); group 1 was retained on 8 L:16D. A week thereafter (i.e. on day 15), groups 1 and 2 birds were sacrificed in the middle of the day and night (n = 5–6 each time). At this time, group 1 birds maintained non-migratory (no fat depot or weight gain) and non-reproductive (small testes) phenotypes, while group 2 birds were photostimulated and exhibited premigratory (initiated fat deposition and weight gain but no *Zugunruhe*) and early reproductive (initiated testis recrudescence) phenotypes. Similarly, groups 3 and 4 were sacrificed in the middle of day and night (n = 5–6 each time) after about 4.5 and 14.5 weeks of long day exposure, respectively. At this time, group 3 birds exhibited migratory and reproductive phenotypes with large adipose fat stores, intense *Zugunruhe* for 7–10 nights and reproductively mature testes (fully recrudesced). Group 4 birds, on the other hand, exhibited postmigratory and gonadally regressed (refractory) states with no fat and lean body mass, daytime activity and reproductively inactive testes. Further details of the experiment and results on phenotypic measurements (activity, body mass, fat score and testis size) are recently published (Trivedi et al., 2014).

2.3. Measurement of plasma levels of free fatty acid, citrate and malate dehydrogenase

Plasma levels of free fatty acid, citrate and mdh enzyme were measured by the quantitative colorimetric citrate determination method using EnzyChrom™ assay kits (free fatty acid, EFFA-100; citrate, ECIT-100; malate dehydrogenase, EMDH-100), as per the manufacturer's protocol (BioAssay System, USA). All assays were carried out in a 96-well plate, and all measurements of optical density were done on the SpectraMax M2 microplate reader (Molecular Devices, USA). Stored plasma samples were allowed to thaw on ice before proceeding for the measurements.

2.3.1. Free fatty acid

Palmitic acid standards were prepared in the final concentration of 1000, 600, 300 or 0 μM by adding the buffer solution. Enzyme A was reconstituted in 120 μl of distilled water. For each well, a working reagent was prepared by mixing 90 μl assay buffer, 1 μl enzyme A, 1 μl enzyme B, 1 μl cosubstrate and 1 μl dye reagent. 10 μl of standards or sample was pipetted out in individual wells, to which 90 μl of working reagent was added. After gentle mixing, the plate was incubated for 30 min at room temperature, and the optical density (OD) was measured at 570 nm wavelength. Free fatty acid concentration was calculated as follows: free fatty acid (μM) = (OD sample − OD blank) / slope of the standard curve.

2.3.2. Citrate

Plasma was diluted five-fold in the distilled water. A 20 μl sample aliquot was used for the assay. For each sample, three reaction volumes of 25 μl per well were used: 20 μl sample + 5 μl standard, 20 μl sample + 5 μl blank, or 25 μl blank (distilled water). A total of 500 μl of 1000 μM citrate standard was prepared by mixing 50 μl of 10 mM standard and 450 μl distilled water, and added to a well as required. Then, 80 μl of working reagent, prepared by mixing 85 μl developer, 1 μl CL enzyme (excluded for sample blanks), 1 μl ODC enzyme and 1 μl dye reagent, was added to each sample well. After the plate was incubated in the dark at room temperature for 15 min, the OD was measured at 570 nm wavelength. The citrate concentration was calculated as follows: citrate (μM) = (OD sample − OD blank / OD standard − OD sample) × (standard / 4) × N; N = plasma dilution