Comparative Biochemistry and Physiology, Part B 152 (2009) 324–331

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Fiber type homogeneity of the flight musculature in small birds

Kenneth C. Welch Jr., Douglas L. Altshuler *

Department of Biology, University of California, Riverside, 900 University Ave., Riverside, CA 92521-0427, USA

ARTICLE INFO

Article history:
Received 7 November 2008
Received in revised form 16 December 2008
Accepted 17 December 2008
Available online 1 January 2009

Keywords:
Avian
Anna's hummingbird
Zebra finch
Fiber type
NADH
Myosin isoform
Wingbeat frequency

ABSTRACT

Studies of medium- and large-bodied avian species have suggested that variation in flight muscle composition is related to differences in flight behavior. For example, slow-twitch or tonic fibers are generally found only in the flight muscles of non-volant or soaring/gliding birds. However, we know comparatively little about fiber composition of the muscles of the smallest birds. Here we describe the fiber composition of muscles from the wings, shoulders, and legs of two small avian species, which also display very high wingbeat frequencies: Anna's hummingbirds (*Calypte anna*) and zebra finches (*Taeniopygia guttata*). All flight muscles examined in both species contained exclusively fast oxidative glycolytic (FOG) fibers. These unique results suggest that fast oxidative fibers are both necessary and sufficient for the full range of flight behaviors in these small-bodied birds. Like all other studied birds, the zebra finch gastrocnemius, a tarsometatarsal extensor, contained a mixture of FOG (27.1%), slow oxidative (SO, 12.7%), and fast glycolytic (FG, 60.2%) fibers. By contrast, the hummingbird gastrocnemius lacked FG fibers (85.5% FOG, 14.5% SO), which may reflect the reduced role of the hindlimb during take-off. We further hypothesize that thermogenic requirements constrain fiber type heterogeneity in these small endothermic vertebrates.

1. Introduction

Most vertebrate skeletal muscles are composed of a diverse assemblage of fiber types (Bottinelli and Reggiani, 2000; Rosser and George, 1986a; Rosser et al., 1996; Schiaffino and Reggiani, 1994). Such diversity of fiber types is indicative of the variety of functions skeletal muscles perform (Bottinelli and Reggiani, 2000). Three general types of twitch fibers, associated with specific subsets of mechanical function, are recognized as components of avian musculature throughout the literature. Slow oxidative fibers (SO) exhibit relatively slow contraction velocities and produce comparatively less force, but are resistant to fatigue and are well suited for slow, repetitive movements or periods of sustained isometric contraction, such as during maintenance of posture. Fast glycolytic (FG) fibers are noted for their rapid contraction dynamics and high level of force generation, while being relatively susceptible to fatigue. These fibers are suited for burst activities requiring great power production. Fast oxidative-glycolytic (FOG) fibers display contraction velocities and capacities for force generation that are intermediate between the other two types and are also relatively fatigue resistant. These motor elements are therefore useful during relatively high frequency activities conducted for longer durations (Bottinelli and Reggiani, 2000). An additional fast-twitch fiber type recognized as being relatively fatigue resistant and containing myosin isoforms distinct from those found in FG and FOG fibers is noted in mammalian skeletal muscle (Schiaffino and Reggiani, 1994). However, this fiber type has not been found in avian muscles (Rosser et al., 1996).

Avian flight musculature has long been of interest to physiologists and morphologists because of the great and diverse functional demands flight behavior places on this locomotory machinery. The pectoralis major powers the downstroke of the avian wingbeat, and the enormous power requirements associated with flight are reflected, at a gross level, in the relatively large size of this single muscle in volant species (Greenewalt, 1962). A great deal of previous work has investigated the diversity of fiber composition of this muscle in relation to the diversity of flight behaviors demonstrated across bird species (Kovacs and Meyers, 2000; Lundgren and Kiessling, 1988; Rosser and George, 1986a; Rosser et al., 1996; Tobalske, 2001; Torrella et al., 1999). Such studies have, for example, generally found that smaller-bodied birds that rely heavily upon powered, flapping flight have pectoralis muscles predominantly containing FOG fibers (e.g. Lundgren and Kiessling, 1988; Rosser and George, 1986a). In contrast, non-volant bird species have pectoralis muscles with comparatively higher concentrations of SO and FG fibers (Rosser and George, 1985; Rosser et al., 1987) and birds that glide or soar have pectoralis muscles with relatively higher numbers of SO fibers (Meyers and Stakebake, 2005; Rosser and George, 1986a,b; Rosser et al., 1994).

There are comparatively fewer studies of the other muscles acting on the wings of birds. Interestingly, even in birds that have pectoralis muscles with homogenous fiber compositions, various wing muscles may contain a diversity of fiber types (e.g. Geyikoglu and Ozkaral, 2000; Kovacs and Meyers, 2000; Marquez et al., 2006; Torrella et al., 1999). Such diversity of fiber composition indicates the potentially

* Corresponding author. Tel.: +1 951 827 3937; fax: +1 951 827 4286.
E-mail address: douga@ucr.edu (D.L. Altshuler).

1096-4959/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2008.12.013

diverse roles these appendicular muscles might play during flight (Dial, 1992).

The fiber type composition of the wing musculature of the smallest birds is largely unknown. Wingbeat frequencies generally scale inversely with body mass, and the very smallest birds, hummingbirds, display a unique form of flight characterized by the highest wingbeat frequencies (Greenewalt, 1962). High wingbeat frequencies require high contraction/relaxation cycling in both power producing, as well as control elements of the flight machinery. Such extremely high operating frequencies, and the potentially long durations over which contraction cycling is sustained, would lead to the prediction that FOG fibers predominate in the wing muscles of these small birds. Previous studies confirm this prediction specifically with respect to the pectoralis and supracoracoideus muscles of hummingbirds (Grinyer and George, 1969; Lasiewski et al., 1965; Mathieu-Costello et al., 1992; Rosser and George, 1986a). However, it is not known how specific muscles in the wing contribute to the control of wing motion and accordingly, we are unable to predict the requirements for force generation, operating frequency, and fatigue resistance in any of the wing muscles. Data pertaining to the fiber composition of these muscles will be beneficial as we gain understanding of their potentially variable functions.

Here, we examine the fiber type composition of the pectoralis, supracoracoideus, and several wing muscles in the Anna's hummingbird (*Calypte anna*) and zebra finch (*Taeniopygia guttata*). These two species are the smallest in mass (<15 g) and display the highest wingbeat frequencies (>25 Hz) (Ellerby and Askew, 2007; Greenewalt, 1962) for which a survey of muscles other than the pectoralis and supracoracoideus has been reported. In addition to shoulder and wing muscles, we examined the fiber composition of the gastrocnemius in both taxa. All three major fiber types have been found in the gastrocnemius muscles of all other birds examined (Marquez et al., 2006; Olson and Olson, 2001; Torrella et al., 1999; Velotto and Crasto, 2004; Viscor et al., 1992; Wada et al., 1999). We anticipated finding the three major fiber types in the hummingbird and zebra finch gastrocnemius and thus chose this muscle for examination in part because we expected it would serve as a positive control with respect to the identification of the three major fiber types. Further, this muscle serves a decidedly different locomotory function compared to the flight muscles and provides information on the nature of fiber composition in each bird species as it relates to other locomotor functions.

2. Materials and methods

2.1. Specimens used in this study

Five adult male Anna's hummingbirds (*C. anna*) and 3 adult male zebra finches (*T. guttata*) were used in this study. Individual birds died during surgery or were euthanized via carbon dioxide asphyxiation or overdose of ketamine/xylazine as part of other studies in our laboratory, and were immediately placed in sealed plastic bags and stored at −20 °C. In cases where birds had received intramuscular injections of ketamine/xylazine, the injected muscles were excluded from use and the contralateral muscles were utilized instead. All birds were of apparent excellent health prior to sacrifice and were fully capable of sustained flight. All vertebrate animal procedures performed were approved by the Institutional Animal Care and Use Committee at the University of California Riverside.

2.2. Hummingbird flight muscle anatomy

Dissections were performed under a Stemi 2000-C stereomicroscope (Carl Zeiss Microimaging, Inc., Thornwood, NY, USA) at magnifications ranging from ×2.5–25. Two hummingbirds were thawed overnight (at 4 °C) and all the bones of the thoracic girdle and forelimb, as well as flight muscles of interest, were then isolated and identified. Work by Zusi and Bentz (1984) describing the myology of the Purple-throated Carib hummingbird was used as a guide for muscle identification in the Anna's hummingbird. Muscle terminology follows that in the "Nomina Anatomica Avium" (Baumel et al., 1979). Skeletal and muscle dimensions were quantified relative to viewing angle under the dissection scope. The position of muscle origin and insertion were noted, and the thoracic skeleton and selected flight musculature were hand-drawn for diagrammatic purposes (Fig. 1).

2.3. Tissue preparation

Whole animal specimens were removed from the freezer and thawed overnight at 4 °C. Whole muscles were then extracted, coated in Tissue-Tek O.C.T. compound (Sakura Finetek USA, Inc., Torrance, CA, USA), and frozen in 2-methylbutane cooled to −160 °C by liquid nitrogen. The following muscles were dissected from each bird: M. pectoralis major (P), M. supracoracoideus (SC), M. biceps brachii (BB), M. tensor propatagialis pars brevis (TPB), lateral section of the M. gastrocnemius (G), and M. triceps brachii scapulotriceps (TBS) and M. triceps brachii humerotriceps (TBH). Both triceps muscles were distinguished prior to removal, and removed, frozen, and analyzed separately. The locations of the wing muscles are shown in Fig. 1. In the case of the M. pectoralis and M. supracoracoideus, the most distal and most proximal several millimeters of muscle were removed (cut perpendicular to the long axis of the muscle) prior to freezing. Similarly, the most distal several millimeters of the M. gastrocnemius (including most of the long tendon) were removed prior to freezing. In the Anna's hummingbird, only two replicates of the M. supracoracoideus, M. biceps brachii, M. triceps brachii (both scapulotriceps and humerotriceps), and M. gastrocnemius muscles were analyzed as there was tissue damage accrued during dissection or during freezing (cracking through the body of the muscle) in the third replicate.

From each available tissue block, transverse sections of 8–12 μm in thickness were cut in a cryostat maintained at −24 to −20 °C. Three to six sections were picked up on each microscope slide (Superfrost® Plus, Fisher Scientific, Pittsburgh, PA, USA), with 18–54 serial sections obtained from each muscle. Slides were either stored in desiccating container at −20 °C for up to 24 h before staining or were stained 2–5 h later. Just prior to staining, all slides were air-dried at room temperature for 1–2 h.

2.4. Fast/slow MHC labeling

Avian skeletal muscle fibers may be distinguished as either slow or fast-twitch by reacting muscle sections with antibodies specific for either slow or fast myosin heavy chain (MHC) isoforms (Rosser et al., 1996). The NA8 monoclonal antibody (Bourke et al., 1995; Cerny and Bandman, 1987) and F30 monoclonal antibody (Crow and Stockdale, 1986; Miller et al., 1985, 1989), each obtained from the Developmental Studies Hybridoma Bank (The University of Iowa, Department of Biological Sciences, Iowa City, IA, USA) were chosen for use in this study as each demonstrates general reactivity with avian slow and fast myosin heavy chain isoforms, respectively.

Immunocytochemical techniques modified slightly from those described by Rosser et al. (1996) and Shear et al. (1988), were used to identify fibers as either fast or slow-twitch. Briefly, sections from each muscle studied were first blocked for 30 min in a solution comprised of 5% goat serum, 1% bovine serum albumin and 5 mM EDTA in PBS (0.02 M sodium phosphate buffer, 0.15 M NaCl, pH 7.2). Sections were subsequently incubated overnight at 4 °C with one of the two primary antibodies diluted in the blocking solution. The NA8 antibody was used at a dilution of 1:50. The F30 antibody was used at a dilution of 1:2. The next day, slides were rinsed with PBS three times for 5 min at a time. Presence of the primary antibody was then visualized by incubating sections for 30 min in the dark at room temperature with a