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**Metabolic cold adaptation in Antarctic fishes: evidence from enzymatic activities of brain**

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**Abstract** To evaluate the concept of metabolic cold adaptation (MCA) in fishes, we compared – in brain, red muscle, and white muscle of Antarctic notothenioid fishes and tropical/subtropical fishes – the activities of two enzymes of ATP-generating pathways, citrate synthase (CS), an indicator of citric acid cycle activity (aerobic metabolism), and lactate dehydrogenase (LDH), an indicator of potential for ATP production through anaerobic glycolysis. Brain was chosen because, unlike locomotory muscle, its metabolic activity is not likely to be influenced by a species’ level of activity or nutritional status, so MCA should be readily observed if present. CS and LDH activities in brain exhibited a high level of MCA, but compensation to temperature was not complete (48% for CS; 46% for LDH). CS and LDH activities in red and white muscle varied widely among species, according to the general level of locomotory activity. The ‘mode of life’-related enzymatic activities in locomotory muscle show that study of MCA at the level of whole organism metabolism is fraught with difficulties and experimental ambiguities. In contrast, the low variation among species within each group in enzymatic activities in brain, and the large differences between groups in CS and LDH activity, show that brain is an excellent study system for evaluating metabolic compensation to temperature.

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**Introduction**

Metabolic cold adaptation (MCA), a concept stating that polar, temperate, and tropical ectotherms exhibit similar metabolic rates at normal habitat temperature, was first proposed by Krogh (1914) and later demonstrated in a classic paper by Scholander et al. (1953) that compared the metabolic rates of terrestrial and aquatic poikilotherms from the Arctic with those of tropical species. They found significantly higher metabolic rates in polar species than in tropical and temperate species when all rates were extrapolated to the low habitat temperature of polar species. MCA, as its name implies, thus regards the metabolic rates of polar species as having undergone an upward adjustment or compensation to offset the effects of low temperature.

MCA received considerable support through a series of studies on whole organism oxygen consumption rates of fishes (Wohlschlag 1960, 1963, 1964; Ralph and Everson 1968; Hemmingsen and Douglas 1970; Morris and North 1984; Torres and Somero 1988a, b) and on metabolism *in vitro* of isolated tissues of Antarctic and temperate fishes (Somero et al. 1968). Despite evidence for MCA in fishes, however, the concept was called into question by Holeton (1974), who questioned the validity of the concept on the basis of both methods and philosophy. Briefly, his arguments focused on the acute experimental methods of Scholander et al. (1953), which in all likelihood resulted in artifactually elevated rates in the polar species they studied, and on the use of Krogh’s ‘normal curve’ for extrapolation of the rates of tropical species to polar temperatures, which would exaggerate the effect of cold adaptation. A further objection brought forth by Holeton (1974) concerned the logic of regarding an elevated standard metabolism as advantageous to cold-water species, when it would only serve to remove energy from other processes such as growth and reproduction. Holeton’s arguments have been developed further by Clarke (1983, 1991, 1998) in a series of thoughtful reviews. Clarke, too, conjectured that
attempts to document MCA, especially through studies using whole organism respiration, are likely to fail. Recent work by Zimmermann and Hubold (1996) has carefully analyzed the influence of mode of life, especially a fish’s activity level, on respiration rate. Their analyses, too, speak of the difficulty of quantifying MCA in species having different activity levels.

Despite the methodological and philosophical criticisms of the concept of MCA, the fact remains that polar fishes perform all of the functions requisite to life at temperatures lethal to tropical and many temperate species. Although some of the adaptations to low temperature found in polar fishes may be strictly concerned with survival per se at low temperatures, for instance, antifreeze thermal hysteresis proteins (Cheng 1998), other adaptations do appear to be related to thermal compensation of metabolic activity, for instance, enzymes with exceptionally high catalytic activities ($k_{cat}$ values) in Antarctic nototohnioid fishes (Fields and Somero 1998). Crockett and Sidell (1990) also report significantly higher activities of enzymes from pathways of aerobic and fatty acid metabolism in tissues of Antarctic fishes than ecotypically similar Temperate Zone species. However, even though there is strong evidence that temperature compensatory adaptations exist at the level of biochemical function, controversy remains as to whether polar species exhibit an overall metabolism higher than would be expected when the rates of temperate and tropical species are extrapolated to colder temperatures.

The present study was designed to shed light on the MCA controversy and on thermal compensation in general by following an experimental strategy that avoided the major pitfalls of much past work on this topic. Rather than examining whole organism metabolism as done in most past studies, we used the activities of enzymes associated with major ATP-generating pathways as a proxy for metabolism of individual tissues. Use of enzymatic activities eliminates effects of (1) the handling stress of direct respiratory measurements, (2) overshoot reactions of fish to abrupt changes in temperature, (3) much of the variation due to differences in nutritional state, and (4) variations in reproductive condition of the experimental animals.

The design of our experiment involved decisions related to appropriate species, enzymes, and tissues to study. We chose to examine a suite of highly cold-adapted Antarctic fishes and warm-adapted tropical/subtropical species to provide a wide range of adaptation temperatures and modes of life (locomotory habit, general activity level, and foraging strategy; see Zimmermann and Hubold 1996). To estimate metabolic activity, we used two enzymes from major ATP-generating pathways: citrate synthase (CS), an indicator of citric acid cycle activity and, therefore, of aerobic ATP-generating potential, and lactate dehydrogenase (LDH), the terminal enzyme in anaerobic glycolysis, whose activity is a strong indicator of locomotory potential (Childress and Somero 1979).

Choice of tissues/organs was especially important for avoiding the pitfalls that might preclude evaluating the extent of MCA in Antarctic fishes. Brain tissue was chosen for studying MCA because it performs the same function in all fishes and its metabolism is less likely to be influenced by the mode of life of a fish. Similar enzymatic activities have been reported in the brains of temperate fishes with diverse locomotory habits (Somero and Childress 1980; Sullivan and Somero 1980; Siebennaller and Somero 1982), which suggests that, at a common adaptation temperature, only minimal variation in the ATP-generating capacity of the brain exists among fishes. Brain enzymatic activity, unlike that of muscle, is also not influenced by a fish’s nutritional condition (Yang and Somero 1992). We also investigated CS and LDH activity in red and white locomotory muscle because these two tissues, white muscle in particular, constitute a large fraction of a fish’s mass, and therefore contribute importantly to whole organism metabolic rate. In summary, by measuring metabolic potential in the brain and muscle tissues of fishes from widely different thermal regimes and having different modes of life, our experimental design allows a rigorous test of the reality of MCA while, at the same time, enabling us to biochemically evaluate the major criticisms by Holstein, Clarke, and others of studies of whole organism MCA.

Materials and methods

Experimental organisms

Eight Antarctic species were studied, all belonging to the suborder Notothenioidei (Table 1). Five of the eight species were in the family Nototheniidae, including the active cryptopelagic hunter Pagophila borchgrevinki, and the less active benthic ambush predators Trematomus bernacchii, Trematomus hansoni, Trematomus nevnesi, and Trematomus pennelli. Three Antarctic icefish (Channichthyidae) were also investigated, two of them benthic, Chaenocephalus aceratus and Chionodraco rastrosomus, and one pelagic, Champsosphaerichthys gunnari. Tropical/subtropical species examined were the active reef-associated damselfish Pomacentrus dorsopunicans, the wrasses Halichoeres bivittatus and Halichoeres radiatus, the chub Kyphosus sp., the lie-in-wait predator Centroprorus undecimalis, and the sluggish freshwater Oreochromis sp.

Collection of specimens

Antarctic fishes were collected in McMurdo Sound during December 1994 and January 1995, and in the Antarctic Peninsula region in January 1996. Specimens of Oreochromis sp. were obtained from a fish market in San Jose, Calif. Tropical/subtropical species were collected on the west coast of Florida and in the Florida Keys in May or June of 1995 and 1996. They were caught with rectangular nets, trawls, or fish traps, or by using hook and line. Fishes were either frozen immediately in liquid nitrogen or brought alive to the laboratory where they were frozen in a cryogenic freezer (−80 °C). All fishes were stored at −80 °C. Loss of enzymatic activity was not observed between fishes immediately analyzed (McMurdo Station, 1995) and fishes stored for 6–12 months and analyzed in the United States (Hopkins Marine Station, Calif., 1996).