Antiquity of the vertebrate pattern of activity metabolism and its possible relation to vertebrate origins

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Vertebrates generally possess well developed capacities for anaerobic metabolism, resulting in formation of lactic acid. Those capacities have traditionally been interpreted in terms of adaptation to hypoxic environments or to special situations such as diving. However, anaerobic metabolism in striated muscle tissue is frequently a major source of ATP utilized during periods of intense activity. The evolutionary significance of anaerobically supported activity has not been discussed, although the interrelationships of capacities for aerobiosis and activity have received considerable attention[1]. We present here evidence that the pattern of activity metabolism utilized by extant species probably dates back to the earliest vertebrates. It is also postulated that the evolution of extensive capacity for anaerobically supported burst activity may have been closely related to the evolution of vertebrates from invertebrate chordates.

Many animals use glycolysis, the anaerobic process by which glucose is degraded to lactic acid. Most invertebrate species resort to significant utilization of glycolysis only in the absence of sufficient environmental oxygen to maintain adequate rates of ATP generation via aerobiosis, or oxidative phosphorylation[2]. However, many vertebrate species rely on prodigious rates of glycolysis for ATP generation during periods of maximal activity[3–6]. Such a pattern of activity metabolism enables many vertebrates to reach 'burst' levels of activity otherwise unattainable if they were solely dependent on aerobic metabolism. Heavy reliance on anaerobic metabolism also has its drawbacks: it is inefficient in utilizing substrate, and is invariably associated with muscle fatigue[7]. In addition, resultant intramuscular lactic acid accumulation and subsequent diffusion of lactate into the cardiovascular system[4] disrupts maintenance of blood and tissue *p*H which may well affect enzymatic activity, protein configuration and so on[8]. This exercise-related *p*H depression may persist in lower vertebrates for several hours, or longer, following cessation of strenuous activity[9].

Although some crustaceans generate moderate quantities of lactate during exercise[10,11], most invertebrates produce little, if any, lactate during maximal activity. For example, during periods of intense activity, the insects are highly dependent on aerobiosis[12] and molluscs anaerobically generate octapene and succinate to supply themselves with sufficient ATP[13]. Hence, the vertebrate capacity for lactate generation during activity should not be considered an adaptation for anaerobiosis *per se*: it is not merely a primitive relict of the occupation of oxygen-depleted environments, but is a specific and relatively unique exploitation of glycolysis to support intense activity.

Lactic acid formation during activity has been demonstrated in many extant gnathostomes (sharks[14], teleost fish[6], amphibians[3], reptiles, mammals[5]). Those data indicate that heavy reliance on lactate formation during activity is widespread among vertebrates. However, the antiquity of this pattern of activity support is still unclear; is it a primitive characteristic dating back to, or even previous to, the origin of vertebrates? To elucidate this question, we investigated patterns of activity metabolism in some living agnathans (Cyclostomata) and the protochordate, amphioxus (Cephalochordata). Morphological evidence indicates long evolutionary isolation of cyclostome and gnathostome lineages as well as independent derivation of both groups from the earliest vertebrates, the ostracoderms of the early Palaeozoic (Fig. 1)[15]. Thus, parsimony dictates that occurrence of a particular trait (burst activity supported by anaerobiosis) in both cyclostomes and gnathostomes might be the result of retention of a primitive characteristic present in the common ancestor of both groups.

Activity physiology in extant protochordates, especially

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Table 1 Whole body lactic acid concentration and blood *p*H before and after activity in two species of cyclostomes and whole body lactic acid concentration before and after activity in amphioxus

	Lactic acid concentration (mg per g tissue)		Blood *p*H			
Species	Rest	Active	Rest	30 min post activity	60 min post activity	180 min post activity
Pacific hagfish (*Eptatretus stouti*)	0.14 ± 0.01 (n = 4)	1.51 ± 0.14 (n = 4)	8.03 ± 0.03 (n = 5)	7.60 ± 0.12 (n = 5)	7.48 ± 0.14 (n = 5)	7.31 ± 0.19 (n = 5)
Pacific brook lamprey (*Lampetrus pacifica*)	0.17 ± 0.01 (n = 5)	1.65 ± 0.18 (n = 7)	7.91 ± 0.04 (n = 5)	7.16 ± 0.06 (n = 5)	—	—
Amphioxus (*Branchiostoma caribaeum*)	0.14 ± 0.03 (n = 4)	0.29 ± 0.04 (n = 8)	—	—	—	—

Post-active depression of blood *p*H probably results from protracted diffusion of lactic acid into the cardiovascular system from striated musculature as well as slow subsequent removal of lactate from the circulatory system. Adult *Lampetra* (36) were obtained by dip net in Oak Creek, Benton County, Oregon, in May 1979. Adult *Eptatretus* (28) were caught in 'fike' traps at a depth of 150 m, 1,000 m off the coast of Coos Bay, Oregon in December 1978. Adult *Branchiostoma* (12) were collected in Tampa Bay, Florida, in December 1979. Animals were maintained in 160-l capacity tanks, situated in thermally controlled rooms set at 12 °C on an 11-h photoperiod. Tank water was kept fully aerated at all times. Experiments were performed 4–6 days after animals were captured. Twelve *Lampetra* (mean mass 6.6 g), 9 *Eptatretus* (mean mass 52.2 g) and 12 *Branchiostoma* (mean mass 0.14 g) were used to determine whole body lactate content either at rest or immediately after maximal activity. Animals were placed individually in a 1 m × 1 m × 0.25 m tank of water equilibrated to 12 °C. They were then stimulated to activity by persistent, noxious but harmless prodding. Reaction of all animals to stimulus was intense, and was assumed to represent maximal activity levels. During stimulation, both *Lampetra* and *Eptatretus* underwent an initial period of vigorous activity lasting 2.5–3.5 min. In both species, this consisted of frantic lateral undulations interspersed with frequent attempts to 'bite' the probe. During the following 1.5–2.5 min, animals appeared to tire but continued to exhibit similar types of behaviour. In addition, mucus was secreted from the slime glands of *Eptatretus* during the entire exercise period. This was removed from the tank approximately every 30 s in such a manner that movements by animals were not hindered. During the initial 10–15 s of the exercise period, *Branchiostoma* underwent intense lateral undulation. Thereafter, they appeared to tire significantly and remained essentially refractory to further stimulus. After 5 min of stimulation, animals were decapitated, homogenized in 0.6 M perchloric acid for 2 min in an Oster blender and total body lactate was measured spectrophotometrically by the method of Bennett and Licht[4]. Similar analyses were also made on unstimulated animals to determine lactate levels for resting individuals. Ten *Lampetra* and 20 *Eptatretus* were used to determine the effect of this activity on blood *p*H. Blood samples of 75 μl were taken in heparinized syringes from five previously undisturbed individuals of each species. Samples were withdrawn from tail incisions in *Lampetra* and from the caudal sinus in *Eptatretus*. Elapsed time between first handling and sample procurement was less than 15 s; struggling by animals was minimal during this time period. The *p*H of the sample was measured immediately with a Radiometer Copenhagen BMS3Mk2 blood micro unit connected to a Radiometer Copenhagen PHM 71 Mk 2 acid-base analyser. The temperature of the electrode was regulated at 12 °C ± 0.5 °C. This measurement was assumed to represent the *p*H of the animals' blood at rest. Different individuals of each species were then stimulated to activity for 5 min as described in the previous experiments. Blood samples were collected and analysed (as described for resting individuals) 30 min after cessation of activity in both species. Additional samples were taken 60 and 180 min after activity in *Eptatretus*.

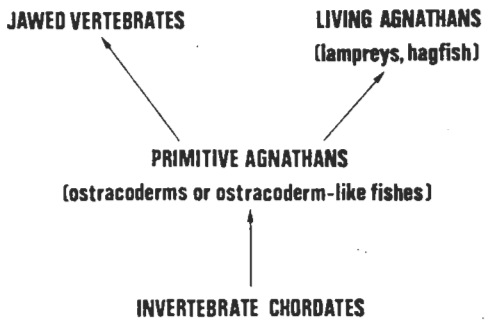

Fig. 1 Phylogenetic relationships of the vertebrates (based on refs 15, 19).

cephalochordates, may well be reflective of activity metabolism in the chordate ancestors of vertebrates. Cephalochordates are thought to resemble closely protovertebrates in many aspects of their morphology and in their sedentary, microphagous filter-feeding lifestyle[16]. Consequently, their activity physiology may also be similar.

Cyclostomes investigated in this study included the Pacific brook lamprey (Petromyzontidae: *Lampetra pacifica*) and the Pacific hagfish (Myxinidae: *Eptatretus stouti*). The Pacific brook lamprey is a small, non-parasitic lamprey inhabiting streams west of the Cascade Mountains of the north-west US. These are strictly freshwater forms that never migrate to marine environments. The Pacific hagfish occurs off the west coast of North America from southern California to south-east Alaska in silty-bottom environments at depths of 18–940 m.

The cephalochordate investigated here was *Branchiostoma caribaeum* (Branchiostomidae). This is a small, burrowing, filter-feeding inhabitant of sandy-bottomed marine environments from Chesapeake Bay to the West Indies.

Both cyclostomes utilized glycolysis extensively during the exercise period (Table 1): lactate concentrations were approximately 10 times greater after 5 min of activity than at rest ($P<0.05$, t-test). In addition, both species experienced a concomitant, protracted decline in all post-active blood pH measurements ($P<0.05$, t-test) (Table 1). Quantities of lactate formed as a result of activity and the accompanying extended post-active decline of blood pH in these species closely parallel previously described modes of activity physiology among gnathostomes[7,9].

Consequently, utilization of the pattern of activity metabolism described here must be of considerable antiquity, perhaps as old as vertebrates themselves. Figure 1 illustrates the generally accepted phylogenetic relationships of the vertebrates. If these relationships are correct, we must assume the pattern of activity metabolism exhibited by living vertebrates is a result of either (1) convergent evolution between cyclostome and gnathostome lineages; or (2) retention of a primitive vertebrate characteristic. Insofar as premise (2) is the most parsimonious, it must be considered the most likely to describe the actual sequence of events.

The implications of that conclusion for the evolution of vertebrates are manifold. For example, parameters of anaerobically supported activity (such as capacity for high levels of burst activity, fatigue, extended post-active blood and tissue pH alteration) have probably vitally affected adaptive radiation of the morphology, physiology and behaviour of virtually all the major groups of vertebrates. In fact, only with the evolution of homeothermy in birds and mammals have aerobic potentials been expanded to the point where, for example, they can support very high levels of activity without consequent fatigue[17].

In contrast, the chordate ancestors of vertebrates may not have possessed such well developed capacities for burst activity as early vertebrates. The cephalochordate studied here was incapable of any more than 10–15 s of intense lateral undulation and generated only minimal quantities of lactate during exercise (Table 1). This pattern of activity physiology is hardly surprising: cephalochordates, like all adult invertebrate chordates, lead a sedentary, filter-feeding existence and seldom, if ever, take any more than the briefest of forays into the water column. Thus, if current interpretations regarding vertebrate origins are correct (see above), one might reasonably postulate a similarly minimal metabolic capacity for intense activity in the chordate ancestors of vertebrates.

Those differences in capacity for activity between invertebrate chordates and the earliest vertebrates might indicate concomitant and interdependent evolution of vertebrate activity physiology and the appearance of the vertebrates themselves. Halstead[18] and others have rightly emphasized that the earliest vertebrates lacked a vertebral column, but differed from invertebrate chordates in that the latter group never exhibits a high degree of cephalization. Accordingly, as described above, all adult invertebrate chordates are at least relatively sedentary, whereas vertebrates are among the most active of the Metazoa. Thus, vertebrate cephalization may have developed in response to selection for increased sensory and locomotor control as a more active lifestyle evolved. We suggest that the expanded vertebrate capacity for burst activity contributed significantly to that active mode of existence. Consequently, evolution of the vertebrate pattern of activity metabolism may well have been a selective factor contributing to cephalization in protovertebrates and the appearance of vertebrates themselves.

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