Comparative Aspects of Hypoxia Tolerance of the Ectothermic Vertebrate Heart

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Abstract This chapter reviews cardiac contractile performance and its regulation during hypoxia/anoxia with regard to cellular metabolism and energy state, in particular hypoxia-tolerant ectothermic vertebrates. Overall the contractile performance of the hypoxic isolated heart muscle varies in a way that relates to the occurrence of hypoxia/anoxia in the natural life of the animal. The hypoxic/anoxic performance of the heart muscle correlates positively with the glycolytic capacity relative to the aerobic capacity, and this performance also tends to be high in hearts having a low aerobic or maximal working capacity. Indirect evidence suggests a particular role for creatine kinase, and that mechanical efficiency may increase in some species. Despite the restricted energy production, hypoxic/anoxic performance is often strongly stimulated by agents such as adrenaline. Frequently, mechanical performance is reduced less by the oxygen lack itself than by factors commonly associated with it, such as increases in extracellular K^+.

1 Introduction

It is generally accepted that survival of vertebrates depends critically on continuous cardiac activity. Cardiac activity and performance may, however, vary considerably between species as well as with endogenous and exogenous conditions, such as temperature, activity, and oxygen availability. This chapter is focused on how myocardial function is affected by severe hypoxia with special focus on ectothermic vertebrate species that display key adaptations to this condition. Examples include diving air-breathers such as turtles, frogs or crocodilians; fish experiencing severely deoxygenated waters; or hagfishes burrowed in carcasses. Hypoxia is often associated with other challenges such as acidosis and elevated extracellular

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potassium concentrations, and the additive effects of such disturbances are therefore also discussed here with regard to myocardial hypoxic performance.

In adult mammals and birds the heart is considered to be almost exclusively aerobic, although myocardial hypoxia may occur under pathological conditions or under special circumstances such as high altitude, diving, burrowing, or parturition (e.g., Jarmakani et al. 1978; Ostadal et al. 1999; Mühlfeld et al. 2005). In contrast, the risk of myocardial oxygen lack is considerably higher in ectothermic vertebrates due to a number of anatomical, behavioral, and environmental reasons. The mammalian and bird myocardium consists of a compact tissue with densely packed muscle cells supplied with blood through an extensively developed coronary system. In contrast, many ectothermic vertebrates have hearts where the atria and parts of the ventricle wall are formed by a spongy myocardium consisting of thin cell bundles forming a network with only a sparse or absent coronary supply. The ventricular wall often consists of two tissue types where an exterior layer of compact tissue, resembling that in ventricles of mammals and birds, surrounds the inner spongy tissue. The proportion of spongy versus compact tissue in the myocardial wall varies according to species and developmental stage. Thus, the compact tissue may constitute as much as 70% in some tuna species, whereas it is about zero in most amphibians and many fish species (Tota 1983; Davie and Farrell 1991; Driedzic and Gesser 1994; Ostadal et al. 1999). Much of the oxygen to the spongy part of the heart is supplied by the blood pumped through the cardiac chamber rather than by a coronary supply. In fish, this is of particular importance as the heart is the last organ before the gills, and the spongy part of the myocardium is therefore perfused by deoxygenated blood returning from the systemic circulation. This has the paradoxical consequence that during intense activity the heart may in fact only receive poorly oxygenated blood although the demand on cardiac activity is increased (Farrell and Clutterham 2003). However, in other situations of severe oxygen deficiency, the demand on the cardiac function is often lower due to a concomitant decrease in the metabolic rate of the whole animal (Driedzic and Gesser 1994; Farrell and Stecyk 2007).

Cardiac output is the product of stroke volume and beat frequency, and both of these parameters are generally easily measured in vertebrates under in vivo, in situ or in vitro conditions. Nevertheless, many studies also use isolated cardiac preparations (e.g., cardiac strips) to address cellular processes. Although such preparations are more remote from the situation in the living animal, they offer some advantages with regard to the control of diffusion conditions and the recording of metabolic parameters. The present review primarily concerns such studies, with the consequence that important aspects of the control and integration of the whole heart, and its function in the living animal, are neglected. In studies of isolated tissue, the effects of challenges such as oxygen deficiency on twitch-force development and relaxation in isolated tissue are often taken as a proxy of the corresponding effect on stroke volume (Fig. 1). A decreased peak force and an impaired relaxation with an elevated resting tension would correspond to an increased end-systolic and a decreased end-diastolic volume, resulting in a reduced stroke volume in the living animal.

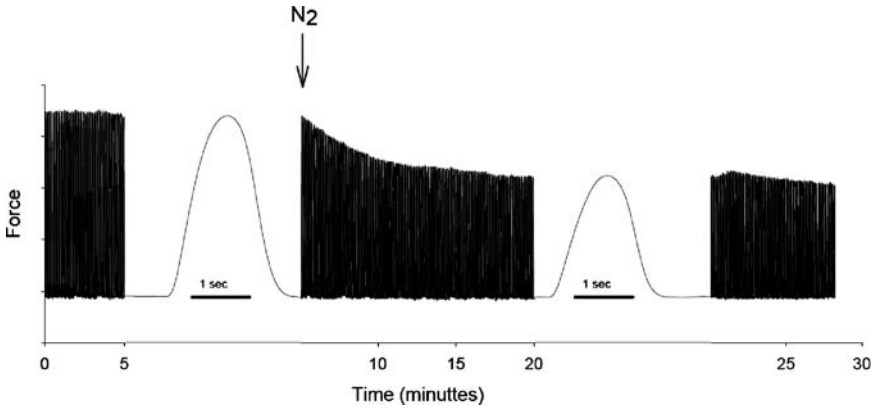

Fig. 1 Representative trace of isometric force development in heart muscle preparation from Red-eared slider (*Trachemys scripta elegans*). Stimulation rate: 0.3 Hz = 18/ min, temp: 20°C, pH: 7.6 (2% CO_2, ~25 mM HCO_3). *Arrow* indicates the transition from oxygenated to hypoxic/anoxic conditions

2 Comparative Hypoxia Tolerance of Cardiac Function

As shown in Fig. 2, the effects of severe hypoxia/anoxia on maximal cardiac contractile force can vary greatly between vertebrate species. Most examples shown in Fig. 2 are from the same laboratory, because even small differences in experimental conditions can markedly influence performance of the oxygen-deficient heart muscle. This is exemplified by cardiac tissue from carp, where force development varies greatly with the total concentration of extracellular bicarbonate–CO_2, or in hypoxic cardiac strips from trout, where adrenergic stimulation or high extracellular Ca^{2+} remove most of the reduction in force generation during severe hypoxia (Gesser 1977; Gesser et al. 1982; Nielsen and Gesser 1983).

Not surprisingly, freshwater turtles are among the best at preserving contractility during anoxia, whereas, for example, mammalian species typically show drastic decreases in twitch force. Also among fish, the best preserved performance seems to occur in species known to have a high hypoxia tolerance such as carp, eel and hagfish (Fig. 2). Thus the risk of an animal species experiencing oxygen lack appears to be a major factor correlating with the anoxic mechanical performance of the isolated heart muscle. Furthermore, hypoxia tolerance seems to be higher in amphibian and reptile species from temperate zones, such as the European viper and the edible frog, whereas tolerance is lower in tropical species such as the marine toad, the alligator and the python (Fig. 2). Other parameters such as gender may also be of importance, as twitch force during severe hypoxia decreased less, or was better recovered upon reoxygenation, in myocardial preparations from female than from male trout and rat (Ostadal et al. 1999; Battiprolu et al. 2007). Moreover, different stocks/lines within a species may vary considerably, as noted for rainbow trout and rat (Baker et al. 2000; Faust et al. 2004).

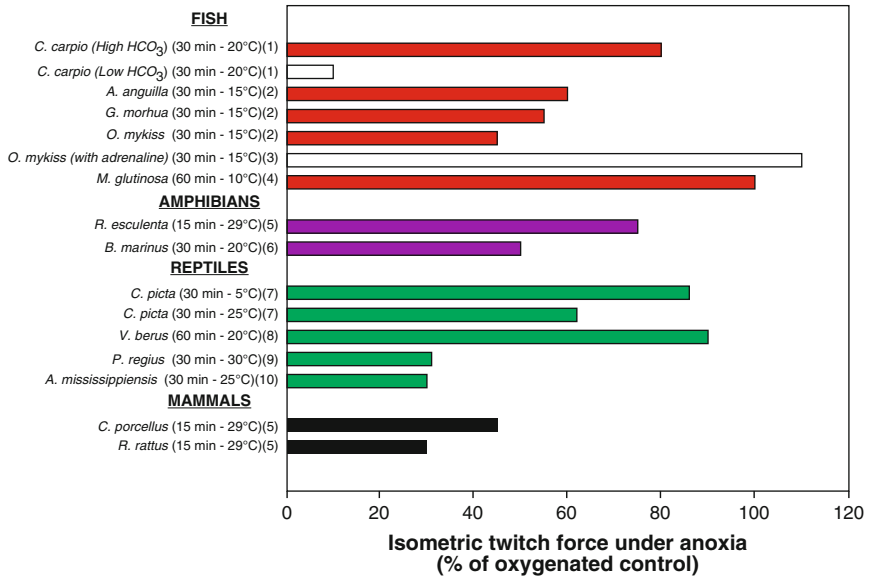

Fig. 2 Anoxic twitch force relative to that under oxygenation (%) in cardiac preparations from: common carp (*Cyprinus carpio*); European eel (*Anguilla anguilla*); Atlantic cod (*Gadus morhua*); rainbow trout (*Oncorhynchus mykiss*); Atlantic hagfish (*Myxine glutinosa*); edible frog (*Rana esculenta*); marine toad (*Bufo marinus*); painted turtle (*Chrysemys picta*); European viper (*Vipera berus*); ball python (*Python regius*); American alligator (*Alligator mississippiensis*); guinea pig (*Cavia porcellus*); rat (*Rattus rattus*). All cardiac preparations are from isolated and electrically paced cardiac strips with the exception of *M. glutinosa* where force is measured with a force transducer coupled to the *in situ* heart preparation. Data for *C. carpio* are shown with high and low $CO_2 - HCO_3$ concentrations (constant pH), and data for *O. mykiss* are shown with and without adrenergic stimulation. All other preparations are run in a physiological solution without pharmacological supplements. Data are obtained from: *(1)* Gesser (1977), *(2)* Hartmund and Gesser (1996), *(3)* Gesser *et al*. (1982), *(4)* Hansen and Sidell (1983), *(5)* Joseph et al. (2000), *(6)* Andersen et al. (2004), *(7)* Overgaard et al. (2005), *(8)* Gesser and Poupa (1978), *(9)* Zaar et al. (2007), and *(10)* Gesser and Wang (unpublished)

3 Cellular Energy Metabolism During Hypoxia/Anoxia

Adenosine triphosphate (ATP) is the primary energy currency in vertebrates. When cellular oxygen availability is high, almost all ATP is provided by oxidative phosphorylation, where 1 mol glucose leads to formation of 26–38 mol ATP (Brand 2005). When ATP production from oxidative phosphorylation is insufficient, ATP can be produced anaerobically by glycolysis, but in this case, 1 mol of glucose units yields only 2 mol ATP with glucose and 3 mol ATP with glycogen as substrate (e.g., Lehninger 1970) (Fig. 3). In addition to the low ATP yield, anaerobic glycolysis is also associated with a fast depletion of energy stores and an accumulation of lactic acid, which causes acidification. Nevertheless, anaerobic metabolism is the sole source of ATP during complete anoxia, but specialized anoxia-tolerant animals

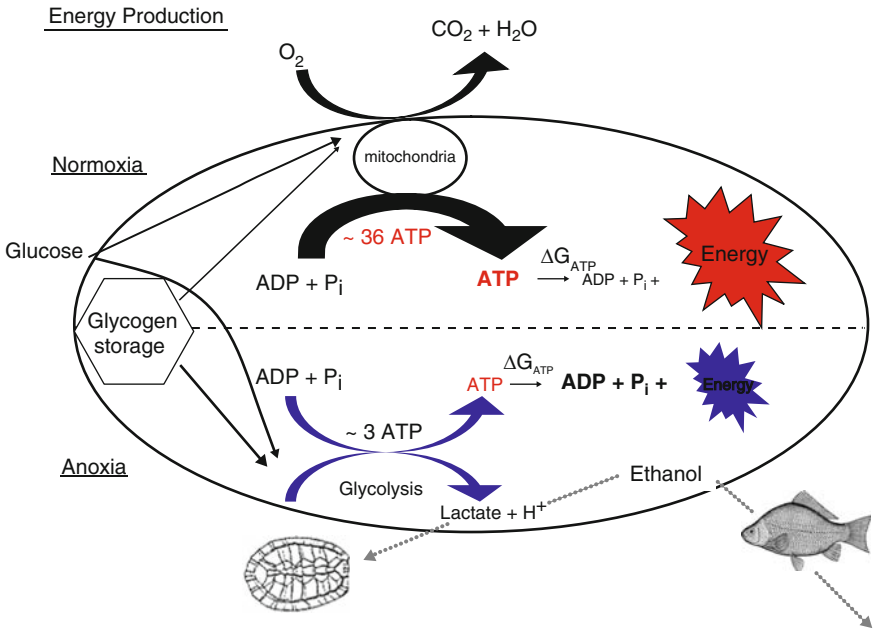

Fig. 3 Schematic presentation of the major effects of anoxia on cellular energy production and energy state: *Top*: normoxic conditions where ~36 mol ATP are produced per mol glucose, and waste products in the form of CO_2 and water diffuse to the extracellular space. Under these conditions, the energetic state (ΔG_{ATP}) is high, with a high ATP/ADP ratio and low levels of free phosphate. *Bottom*: anaerobic conditions where fermentation of glucose only produces 2–3 mol ATP per mol glucose. The waste product lactic acid accumulates intra- and extracellularly, causing intra- and extracellular acidification. However, the anoxia-tolerant crucian carp can excrete the waste products in the form of ethanol, and anoxia-tolerant turtle species can alleviate the acidification by large buffer reserves in the shell and bone. During anoxia, energy state (ΔG_{ATP}) decreases, and the free phosphate level increases

can attenuate the associated adversities. Thus, the ability of freshwater turtles and crucian carp to survive anoxia relies, in part, on their extraordinary ability to handle anaerobic waste products. Freshwater turtles utilize the large buffer capacity of bone and shell to attenuate acidification due to lactic acid (Jackson 2000, 2002), while ethanol, which is excreted through the gills, is the anaerobic end-product in crucian carp (Van Warde 1991) (Fig. 3).

3.1 Metabolic Depression of the Whole Animal and the Heart

The cardiac demand varies with whole body energy turnover, which is typically low in vertebrates enduring long-term anoxia. According to Farrell and Stecyk (2007), a low cardiac power output is attained through different strategies. Thus, some anoxia-tolerant species, such as crucian carp and hagfish, have an inherently low

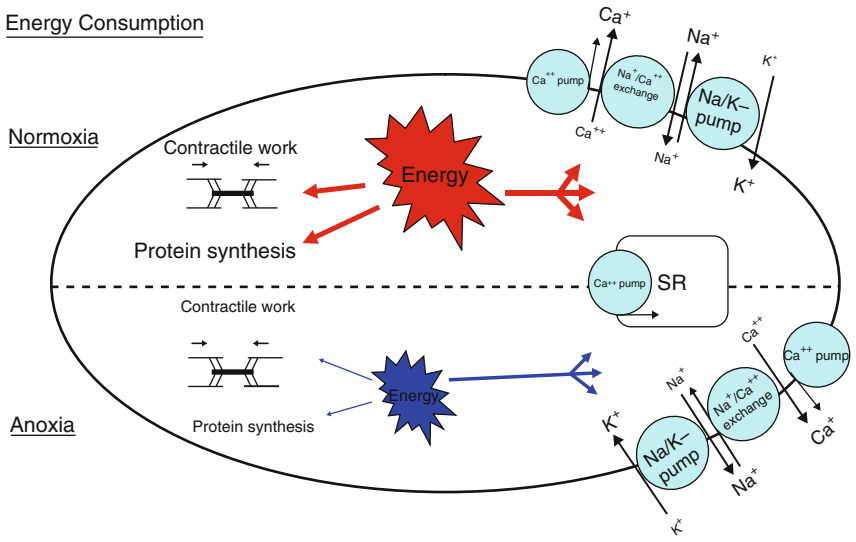

Fig. 4 Schematic presentation of the effects of anoxia/hypoxia on cellular energy consumption in cardiac cells. *Top*: normoxic conditions where energy is predominantly used for protein synthesis, contractile work, and ion motive pumps such as the Na^+/K^+ ATPase and the Ca^{2+} ATPase. The activity of these pumps maintains transmembrane ion homeostasis, which is essential for the excitability and the ability to maintain Ca^{2+} transport during excitation–contraction. *Bottom*: Anaerobic conditions are associated with a general metabolic depression due to translational arrest, spike and channel arrest (*see text*)

routine cardiac power output, while, for example, freshwater turtles severely depress cardiac power output during anoxia (Farrell and Stecyk 2007). This is generally in accordance with whole body metabolic rate, where the crucian carp maintains activity and displays a moderate down regulation of metabolic rate during anoxia (Nilsson and Renshaw 2004), whereas anoxic freshwater turtles reduce whole body metabolism by 80–90% (Jackson 1968). Furthermore, this reduction is amplified at low temperature during winter hibernation, when the turtles may enter a coma-like state (Lutz and Nilsson 1997; Storey and Storey 1990; Boutilier 2001; Jackson 2002).

In addition to an upregulation of anaerobic energy production, the transition from oxygenated conditions to anoxia involves a reorganization of cellular energy-consuming processes (See Fig. 4), which may depress overall metabolic requirements (Hochachka 1986; Driedzic and Gesser 1994; Hochachka et al. 1996; Lutz and Nilsson 1997; Boutilier and St-Pierre 2000; Boutilier 2001). Overall, protein synthesis is reduced dramatically during anoxia, although synthesis of a few specific proteins, including heat shock proteins, may be upregulated in anoxic turtles (Chang et al. 2000). Protein synthesis normally accounts for a large part of cellular energy turnover so "translational arrest" renders a larger proportion of the available ATP for ion pumping (Hochachka et al. 1996). Depending on the tissue type, ion motive pumps may account for 25–75% of total metabolism during normoxia (see Hochachka et al. 1996; Boutilier and St-Pierre 2000; Boutilier 2001).

However, since overall ATP-turnover is reduced by more than 75% in some species the activity of ion motive pumps is obviously bound to be reduced, despite the fact that an enlarged fraction of ATP is available to ion motive pumps during anoxia in turtles (Hochachka et al. 1996; Boutiler and St-Pierre 2000). The overall reduction in activity of ion motive pumps affects ionic gradients and consequently cellular integrity, unless it is accompanied by a simultaneous reduction in the passive transmembrane ion transport that is associated with electrical activity, membrane co- and countertransport and leakage. Such reductions do occur, and they have been referred to as "channel arrest" and "spike arrest". Channel arrest is caused by a reduction in the number and permeability of the membrane ion channels. Channel arrest has been reported for Na^+, K^+ and Ca^{2+} channels in turtle brain, and for the Na^+ channel in hepatocytes (see Hochachka et al. 1996; Boutilier and St-Pierre 2000; Jackson 2000). Spike arrest, in which the electrical activity of excitable tissue is reduced, is afforded by reduced channel activity, and also by increased levels of inhibitory neuro-transmitters and a decreased neural activity (Hochachka et al. 1996; Lutz and Nilsson 1997; Boutilier and St-Pierre 2000; Boutilier 2001; Jackson 2002). Despite the marked reorganization of energy-consuming processes, a new steady state is not necessarily attained during long-term anoxia. Thus, continued anoxic exposure is associated with a gradual increase in extracellular potassium ($[K^+]_0$) in turtles, where $[K^+]_0$ increases from $\sim$3 mmol l^{-1} to values exceeding 10 mmol l^{-1} in both painted turtle and snapping turtle (Jackson and Ultsch 1982; Ultsch et al. 1999; Reese et al. 2002) (See Fig. 4). It should also be noted that channel arrest does not always occur during long-term anoxia, as exemplified by the heart of crucian carp (Paajanen and Vornanen 2003).

3.2 Aerobic Metabolism and Hypoxia

The ability to sustain cardiac function during hypoxia depends not only on the glycolytic capacity but also on the ability to maintain oxidative phosphorylation at low oxygen availabilities. This aspect seems somewhat neglected, possibly due to experimental problems relating to the variation in the degree of hypoxia that different cells will experience in multicellular preparations. The maintenance of aerobic ATP production includes the cellular means for supplying mitochondria with oxygen, as well as the ability to maximize the degree of coupling (i.e., the P/O ratio – ATP per oxygen molecule). In recent years, the picture of the regulation of this coupling has changed considerably, as reviewed by Brand (2005). Thus, in both endo- and ectothermic vertebrates, the P/O ratio seems more variable and typically below the value of 6 provided by the standard biochemical textbook. For example, a low activity level in a tissue well supplied with oxygen may result in a reduced P/O ratio mainly because of a proton leak through the inner mitochondrial membrane (Brand 2005). Hypoxia can augment the P/O ratio as it reduces the mitochondrial proton gradient and thereby the proton leak, as well as increasing the substrates for oxidative phosphorylation (ADP and inorganic phosphate) (Gnaiger 2001). Moreover,

an increased efficiency of ATP consuming processes would favor energy balance during severe hypoxia, especially in organs such as the heart, which maintain function during anoxia. Indeed we have observed an apparent decrease in the cost of contraction during hypoxia in ventricular strips of freshwater turtle, which may be related to an improved P/O ratio. However, the underlying mechanisms for this apparent improvement of energy economy are still unclear, and a reduced expenditure to noncontractile processes may also be involved (Overgaard and Gesser 2004). However, in a direct study of the cost of cross-bridge formation in mammalian heart muscle, there was no evidence for an improved efficiency during hypoxia, and cost of cross-bridge formation actually tended to increase in hypoxia-sensitive species (Joseph et al. 2000).

3.3 Enzyme Complement and the Relation Between Contractility and Energy State

Glycolytic ATP delivery is particularly important during hypoxia/anoxia, and it has been suggested that a high glycolytic enzymatic capacity relative to the aerobic enzymatic capacity is an indicator of the ability to maintain anaerobic cardiac performance. Indeed, a comparison of isolated ventricular preparations from different teleost species provided a positive correlation between the maintenance of hypoxic contractility and the ratio of the glycolytic to the aerobic capacity assessed respectively by activities of pyruvate kinase and cytochrome oxidase recorded in vitro (Gesser and Poupa 1974) (Fig. 5). Similarly, heart muscle from freshwater turtle, crucian carp and hagfish (*myxine glutinosa*), which are known for the ability to maintain contractility during anoxia, displayed the highest ratios of pyruvate kinase to cytochrome oxidase among 13 vertebrate species (Christensen et al. 1994). However, the high ratio found in anoxia-tolerant species is to a large extent due to a low aerobic capacity (a low cytochrome oxidase activity) rather than to a high glycolytic capacity (a high pyruvate kinase activity) (Christensen et al. 1994). Accordingly, a comparison of heart muscle from 15 vertebrate species shows that the glycolytic capacity first increases and then levels off as the aerobic capacity increases (Driedzic et al. 1987). Thus, hypoxic tolerance seems predominantly to relate to a low energy turnover, rather than to a high glycolytic flux per se (Driedzic et al. 1987; Overgaard and Gesser 2004; Farrell and Stecyk 2007).

Glycolysis is also of importance in the oxygenated heart where it protects contractility and electro-mechanical coupling in a way not necessarily reflected in metabolic capacity data (Lorenz and Paul 1997; Gesser 2002; Kockskamper et al. 2005; Farrar et al. 2006, Battiprolu et al. 2007). In heart muscle from both turtle and rainbow trout glycogen appears to be the main substrate during severe hypoxia (Reeves 1963; Battiprolu et al. 2007), whereas access to glucose seems to improve contractility during oxygenation (Battiprolu et al. 2007). The situation may, however, vary with species as well as conditions. Hence, in the anoxic turtle heart a shift towards glucose utilization appears as the contractile performance approaches its maximum (Reeves 1963), and the cardiac glucose uptake increased fivefold during

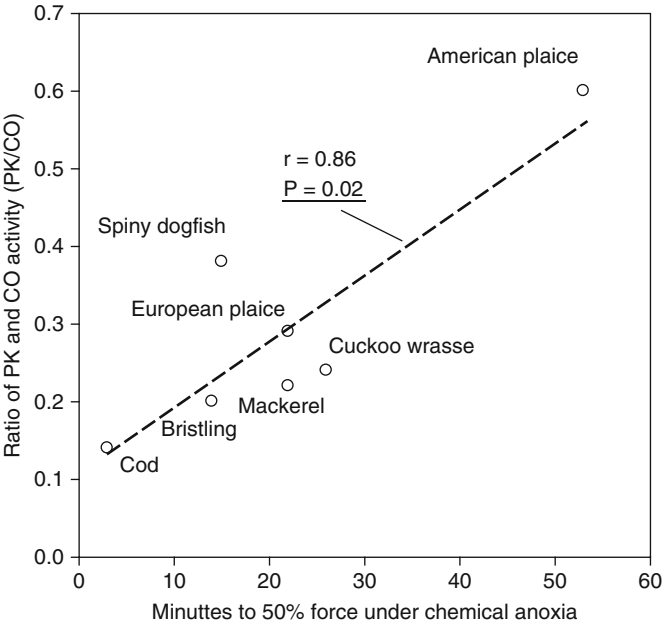

Fig. 5 Relationship between the relative glycolytic capacity and hypoxia tolerance in seven species of marine fish: cod (*Gadus morrhua*), bristling (Sprattus sprattus), mackerel (*Scomber scomber*), spiny dogfish (Squalus acanthias), European plaice (Pleuronectes platessa), cuckoo wrasse (*Labrus ossifragus*), and American plaice (*Hippoglossoides platessoides*). The relative glycolytic capacity is calculated from the ratio of pyruvate kinase and cytochrome oxidase activity (PK/CO). Here it is assumed that pyruvate kinase activity is a proxy for glycolytic capacity, and cytochrome oxidase activity is a proxy for oxidative capacity. Data are from Gesser and Poupa 1974

hypoxia in sculpins in which the receptors for acetylcholine and adenosine were blocked (MacCormack and Driedzic 2007).

Another remarkable feature of the enzymatic complement of the heart muscle from hypoxia-tolerant species is the high activity of creatine kinase (Christensen et al. 1994). Creatine kinase may be crucial due to its role in the cellular energy distribution relating to cellular compartmentation. Accumulating evidence indicates that the cell contains separated units, in which processes consuming and producing energy are tightly coupled (e.g., Saks et al. 2006; Birkedal and Gesser 2006). Thus, the creatine kinase reaction functions as a "spatial buffer" because it facilitates the maintenance of sufficient diffusion rates of ATP and ADP between ATP-consuming and -producing regions within the myocyte. A high creatine kinase activity will therefore limit local reductions in energy state at the ATPase sites. Similarly, the creatine kinase reaction can also counteract local acidification at the ATPase sites as the rephosphorylation of ADP to ATP binds one H^+ (Wallimann et al. 1992). Indirect evidence for the significance of both creatine kinase and glycolysis under hypoxia was the elevated myocardial activities of both creatine kinase and pyruvate kinase in turtles that had been acclimated to anoxic conditions (Birkedal and Gesser 2004).

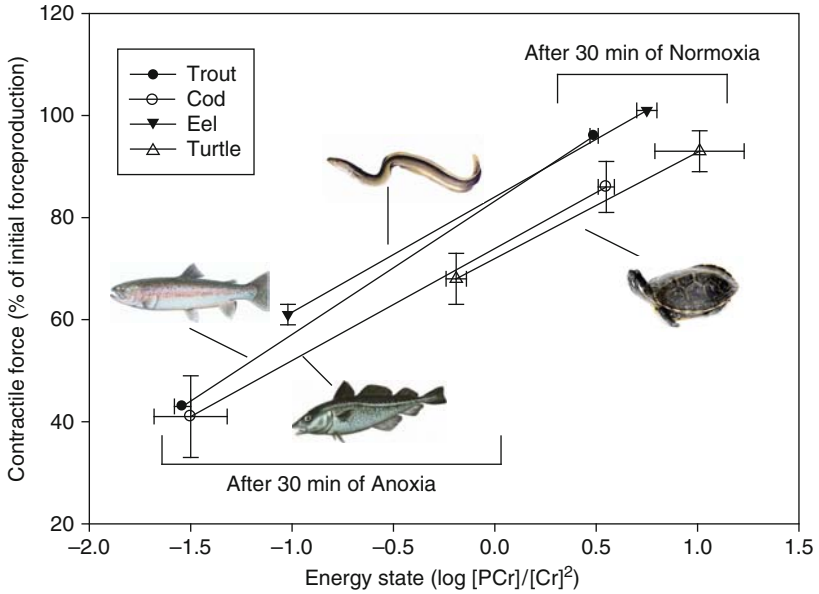

Fig. 6 Relationship between contractile force and energy state in ventricular preparations from four vertebrate species (cod, eel, trout and turtle). Data are from Hartmund and Gesser, 1996. The ventricular preparations were exposed to either 30 mins of normoxia (*right side*) or 30 min of anoxia (*left side*). Force is expressed relative to that produced at the onset of the experiment, and the energy state is approximated by the ratio of $\log([PCr]/[Cr]^2)$ (*see text for details*). The figure illustrates that the hypoxia-tolerant species (turtle and eel) are just as sensitive to decreases in energy state as the hypoxia-sensitive species. However, the tolerant species are characterised by the ability to maintain energy state significantly higher during anoxia, and therefore they retain the ability to produce contractile force under these adverse conditions

4 Cellular Energy State and Contractile Force

When cellular respiration is impaired by removal of oxygen or application of cyanide (chemical anoxia), the myocardium typically responds with a decrease in force production and/or contraction frequency. Resting tension (i.e., force during diastole) either increases or stays unchanged, as exemplified in Fig. 1. These changes follow upon decreases in cellular energy state, ΔG_{ATP}. Indeed, contractility during severe hypoxia correlates closely with the cellular energy state as exemplified in Fig. 6. Thus, in a study of four vertebrate species, contractile force and the estimated ΔG_{ATP} fell together along a regression line that did not differ significantly for turtle, eel and cod, whereas the regression line for trout tended to be steeper (Hartmund and Gesser 1996).

Energy state (ΔG_{ATP}) is defined as the free energy released by hydrolysis of ATP to ADP and P_i:

$$\Delta G_{\mathrm{ATP}} = \Delta G^{0}_{\mathrm{ATP}} - RT \ln \frac{[\mathrm{ATP}]}{[\mathrm{ADP}][P_{\mathrm{i}}]}.$$

The calculation of ΔG_{ATP} is based on the free concentrations of the reactants and, except for ADP, they can for example be measured by NMR. The free concentration of ADP can not be directly measured by available techniques, and ΔG_{ATP} is therefore often assessed using the creatine kinase reaction:

$$P\mathrm{Cr} + \mathrm{ADP} + \mathrm{H}^{+} \rightarrow \mathrm{Cr} + \mathrm{ATP}.$$

Assuming that the creatine kinase reaction is close to equilibrium; that [ATP], [*P*Cr] and [Cr] are "free"; and that the bulk of cellular P_{i} comes from the hydrolysis of creatine phosphate, the term $\frac{[\mathrm{ATP}]}{[\mathrm{ADP}][P_{\mathrm{i}}]}$ may be approximated by the expression $K\mathrm{eq} \times \frac{[P\mathrm{Cr}]}{[\mathrm{Cr}]^2}$ (Meyer 1988).

The creatine kinase reaction is strongly directed to ATP formation as shown by the apparent equilibrium constant, *K*eq, being >100 (Teague et al. 1996). It is clearly important, therefore, not to evaluate the energy state from [ATP] alone, as $\frac{[\mathrm{ATP}]}{[\mathrm{ADP}][P_{\mathrm{i}}]}$ determines the amount of energy released by ATP hydrolysis. Hence, the ATP concentration may decrease by only 10–20% during anoxia while the energy state may be severely reduced through large fractional increases in "free" [ADP] and phosphate (Wasser et al. 1990; Jackson et al. 1995; Hartmund and Gesser 1996; Overgaard and Gesser 2004).

Since the contractility of heart muscle from different vertebrates seems to be affected similarly with decreasing energy state (Fig. 6) (Hartmund and Gesser 1996), it can be argued that it is the maintenance of energy state rather than the tolerance to a low energy state that differs between anoxia-tolerant and -intolerant species (van den Thillart 1989; Hartmund and Gesser 1996). As long as the energy consumption exceeds the energy production (See Figs. 3 and 4) in the hypoxic myocardium, the energy state will decrease while ADP and phosphate will increase. Increases in ADP and phosphate, both of which are important substrates in glycolysis and oxidative phosphorylation, will stimulate the anaerobic metabolism (Pasteur effect) so that a balance between energy-consuming and -producing processes is approached (Hardie 2000; Andrienko et al. 2003). Thus, the truly hypoxia-tolerant vertebrates are not necessarily characterized by a high anaerobic capacity, but rather by the ability to match energy production and consumption at a relatively high energy state (Figs. 4, 5, and 6) (van den Thillart 1989; Wasser et al. 1990; Buck et al. 1998; Overgaard and Gesser 2004).

Cardiac contractility and its regulation are probably less affected by a lowering of the energy state itself than by factors associated with it, such as increases in free inorganic phosphate and decreases in pH due to a net hydrolysis of creatine phosphate and lactic acidosis (Fig. 7). Both these changes lower myofibrillar Ca^{2+} sensitivity (Driedzic and Gesser 1994; Fukuda et al. 2001; Crampin and Smith 2006) and inhibit the power stroke step in the myosin–actin cycle (Cooke and Pate 1985; Godt and Nosek 1989; Westerblad et al. 2002). It is worthy of note that the cellular content of phosphocreatine and thus the release of free phosphate may

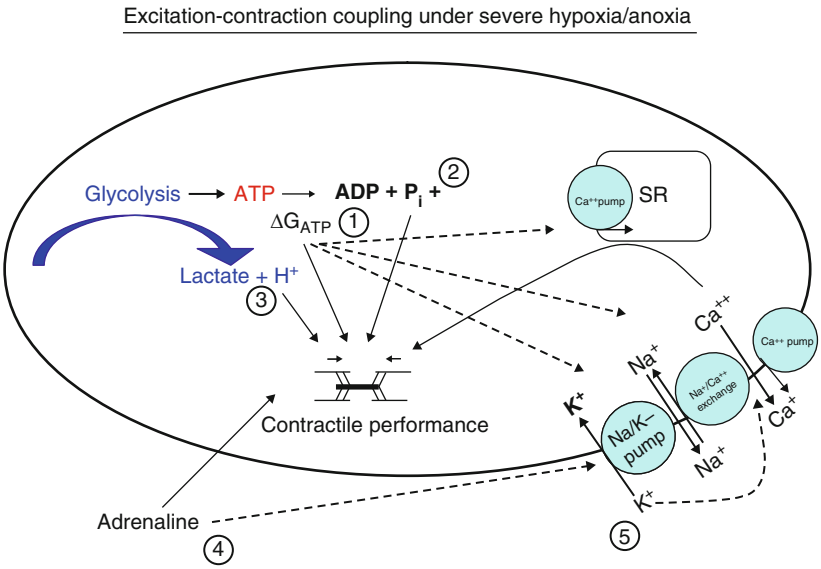

Fig. 7 Direct and indirect effects of hypoxia on excitation–contraction coupling in cardiac cells. A decrease in energy state under hypoxia may directly affect the ATP hydrolysis associated with the actin–myosin interaction, or it may act indirectly by affecting the activity of ion-motive pumps in the sarcolemma or sarcoplasmatic reticulum (*1 in circle*). Increased concentrations of ADP and P_i associated with hypoxia will also directly reduce the power stroke of the actin myosin interaction (*2 in circle*). The increased concentration of protons associated with anaerobic metabolism will also reduce the contractile performance through competitive binding of hydrogen ions to the Ca^{2+} binding sites of troponin C (*3 in circle*).These effects may be counteracted by a hypoxia-induced release of adrenaline, which increases the ATP regeneration and the Ca^{2+} transient, and stimulates Na^+/K^+ ATPase and the maintenance of transsarcolemmal ion balances (*4 in circle*). Nevertheless, a progressive increase in K^+ may develop during long-term anoxia in turtles in particular, and this will depolarise the membrane and shorten the ventricular action potential, whereby Ca^{2+} influx and contractility are depressed (*5 in circle*)

differ among species. For instance, the phosphocreatine store in heart tissue from the hypoxia-tolerant freshwater turtle is relatively small compared to other ectothermic vertebrates (Christensen et al. 1994). As a consequence of the low phosphocreatine stores, a decrease in ΔG_{ATP} will only result in a relatively small release of phosphate. Furthermore it seems that the impact of phosphate on contractility is reduced at decreased cellular energy states in the freshwater turtle myocardium (Jensen and Gesser 1999).

5 Cardiac Excitation–Contraction (E, C) Coupling

The vertebrate heart muscle works as a syncytium, i.e., the contractile proteins of all its cells are activated simultaneously by elevations of the cytosolic Ca^{2+} activity of which the magnitude and duration determine the degree of activation. The Ca^{2+}

activity is governed mainly by the membrane ion transport mechanisms indicated in Fig. 7. An inward Na^+ current drives the membrane potential to positive values and opens L- type Ca^{2+} channels through which Ca^{2+} enters the cell down its electrochemical gradient. This inward current is balanced by an outward K^+ current so that the membrane potential stabilizes at a plateau. Here the Na^+–Ca^{2+} exchange (Fig. 7) may contribute to the Ca^{2+} influx due to a reversal of the combined gradients for Na^+ and Ca^{2+} (Vornanen 1999; Hove-Madsen et al. 2003; Galli et al. 2006b). The action potential is terminated when an increased outward K^+ current repolarizes the cells and closes the L-type Ca^{2+} channels (Bers 2002). The Ca^{2+} entered across the sarcolemma elevates the cytosolic Ca^{2+} activity and causes the Ca^{2+} transient, which activates the contractile proteins either alone or after having been amplified by a Ca^{2+} induced Ca^{2+} release from the sarcoplasmatic reticulum (SR) (Fig. 7). The SR provides most of the activator Ca^{2+} in the mammalian and bird cardiac cells, while the Ca^{2+} entered across the sarcolemmal typically predominates in ectothermic vertebrates, and in many ectothermic species it is uncertain if the SR contribution is of any significance (Tibbits et al. 1990; Thomas et al. 1996; Bers 2002). The Ca^{2+} activating contractility enters the cytosol passively along its electrochemical gradient. In contrast, its removal and in turn relaxation depend on energy-requiring processes such as the Na^+–Ca^{2+} exchanger and Ca^{2+}–ATPases in the sarcolemma and the SR membrane (Fig. 7) (Bers 2002). Here the $Na^+ - Ca^{2+}$ exchanger is driven by the Na^+ gradient provided by the Na^+/K^+- ATPase. Mainly because of the energy requiring Ca^{2+} removal, the E–C coupling has been estimated to consume up to 20–30% of the energy supporting the contractile activity (Kammermeier 1997; Gibbs 2003).

The E–C coupling is the main determinant of mechanical activity. Studies of action potential and membrane currents in heart muscle from ectothermic vertebrates such as crucian carp, goldfish and freshwater turtle suggest that the influence of hypoxia on the E–C-coupling is complex and species-dependent. As in the case of endothermic vertebrates (Vleugels et al. 1976), the action potential was shortened under hypoxia in myocardium from goldfish. This shortening probably reduces activation of the contractile apparatus and has been ascribed to an opening of sarcolemmal ATP dependent potassium channels (K_{ATP}) mediated by nitric oxide, NO, and c-GMP following a lowered ATP/ADP ratio (Cameron et al. 2003; Chen et al. 2005).

A lowered energy state is most probably the prime cause for the opening of the K_{ATP} channels in both the sarcolemma and the inner mitochondrial membrane (Noma 1983; MacCormack and Driedzic, 2002; Cameron et al. 2003; Chen et al. 2005). However, the role of the mitochondrial K_{ATP} channels is not settled (e.g., Dröse et al. 2006). It has been suggested that they are involved in increasing the viability of hypoxic myocardial cells (Chen et al. 2005), and mitochondrial K_{ATP} channels have also been linked to hypoxic preconditioning (Yellon et al. 1998). In yellowtail flounder myocardium, anoxia induced an increase in twitch force, which was abolished in the presence of diazoxide, an inhibitor of the opening of mitochondrial K_{ATP} channels (MacCormack and Driedzic 2002). When mitochondrial K_{ATP} channels open, they allow a flux of K^+ into the mitochondrial matrix, which

depolarizes the mitochondria and causes Ca^{2+} release (Holmuhamedov et al. 1998). Thus, it has been suggested that mitochondrial Ca^{2+} release occurs during hypoxia and/or acidosis in ectothermic vertebrates (Gesser and Poupa 1978; Bowser et al. 1998; MacCormack and Driedzic 2002), and such a release may also be behind the finding (Vornanen 1999) that the action potential of ventricle cells from the anoxia-tolerant crucian carp was prolonged instead of shortened, upon inhibition of the cell respiration. This prolongation appeared to be due to an inward current through the Na^{+}–Ca^{2+} exchanger following a release of some intracellular Ca^{2+} store.

In turtles kept under prolonged anoxia at 21°C, the duration of action potential is prolonged, and the density of the inward sarcolemmal Ca^{2+} current decreased in ventricular but not atrial cells. No effects appeared for cells from turtles subjected to prolonged anoxia at 5°C, which is closer to the temperature experienced by the turtles during winter hibernation in ice-covered ponds. Low temperature itself, however, entailed a substantial increase in the resting membrane potential to less negative values; a several-fold prolongation of the action potential; a decreased density of the calcium current through the L-channels; and a lowered spontaneous contraction rate. These changes may represent a preacclimation to the poor availability of oxygen and energy during hibernation. Thus, the prolongation of the action potential seems to correlate with a decreased frequency of spontaneous contraction and thus with a lowered energy demand (Stecyk et al. 2007). The functional significance of these effects associated with hypoxia on the duration of action potential and on ion currents is unclear, although they may protect the cellular energy state by lowering both twitch-force development and heart rate, and the energy expenditure of both myosin ATPase and ion transport (Stecyk et al. 2007).

6 Factors Influencing Cardiac Contractility During Anoxia

Decreased ATP production in the hypoxic myocardium may reduce contractility directly through changes in energy state, $[P_i]$ or [ADP], but hypoxic/anoxic conditions may also affect contractility indirectly through changes in the intra- and extracellular compartments. Increased $[H^{+}]$ associated with lactic acid formation will, for example, directly influence contractility, and acidification may also have indirect effects by increasing plasma Ca^{2+} in some ectothermic vertebrates (Ruben and Bennett 1981; Jackson and Heisler 1982). In both turtle and trout, severe hypoxia is associated with a large increase in circulating catecholamines, which may enhance contractility (Wasser and Jackson 1991; Perry and Reid 1994). Finally, prolonged metabolic depression in hibernating freshwater turtles may increase extracellular potassium, which will depolarize the plasma membrane and shorten the cardiac action potential (Ultsch and Jackson 1982; Nielsen and Gesser 2001; Overgaard et al. 2005). Hyperkalemia may also arise during intense activity, which for the teleost heart may entail hypoxia. Thus, hypoxia or anoxia is associated with a number of changes in the intra- and extracellular compartments that may alter

mechanical activity in the heart. The isolated and combined effects of these intra- and extracellular disturbances have recently been reviewed with respect to the freshwater turtle myocardium (Overgaard et al. 2007), and below we briefly discuss the general influence of these factors on myocardial contractility.

6.1 Acidosis

Anoxia is typically associated with a progressive extra- and intracellular acidosis (Allen et al. 1985; Wasser et al. 1990; Jackson et al. 1995). Acidosis affects several parts of the E–C coupling and is generally believed to exert negative inotropic effects on mammalian cardiac muscle which relate to a depression of myofilament Ca^{2+} responsiveness (Orchard and Kentish 1990; Crampin and Smith 2006). This decrease in Ca^{2+} responsiveness is caused by the competitive binding of hydrogen ions to the Ca^{2+} binding sites of troponin C (see Crampin and Smith 2006).

Acidosis markedly decreases cardiac twitch force in most fish, while it induces more modest effects in many air-breathing ectotherms such as, for example, freshwater turtles (Poupa et al. 1978; Gesser and Poupa 1983; Overgaard et al. 2005; 2007). In a few species, such as flounder and the marine toad, acidosis has even been reported to exert positive inotropic effects (Gesser and Jørgensen 1982; Andersen et al. 2004). Thus, it has been suggested that the negative effects of acidosis are counteracted by an increase in intracellular Ca^{2+}, possibly from mitrochondrial stores (Gesser and Poupa 1978; Driedzic and Gesser 1994). The effects of acidosis may also be influenced by their interaction with other factors; thus, lactic acidosis did not influence contractility in ventricular preparations from freshwater turtles at 25°C, but it did lead to a ~30% reduction in twitch force at 5°C. Hence, it is possible that acidosis impairs contractility more during overwintering than at higher temperature. Furthermore, anoxia and acidosis in combination typically reduce heart muscle performance much more than they do when acting separately. Thus, severe acidosis may inhibit glycolysis and thereby contribute to a reduction in cellular energy state. (Wasser et al. 1990; Jackson et al. 1995; Shi and Jackson 1997; Shi et al. 1999; Bobb and Jackson 2005). Finally, it should be noted that the type of acidosis is of importance: metabolic acidosis seems to have less dramatic effects on contractility than respiratory acidosis (Driedzic and Gesser 1994).

6.2 Hyperkalemia

Anoxia and hypoxia may be linked to increased levels of extracellular potassium ($[K^+]_0$) in some species. Hyperkalemia may occur during intense activity where, for example, teleost hearts may also experience hypoxia. However, the detrimental effects of hyperkalemia are mainly of relevance for animals such as freshwater turtles, where increasing $[K^+]_0$ reflects an inability of the Na^+/K^+ pump to maintain a normal transcellular cation distribution during long-term anoxia (Jackson and Ultsch 1982, Ultsch et al. 1999; Reese et al. 2002). In these situations $[K^+]_0$

may increase above 10 mM, which will severely depress twitch force (Nielsen and Gesser 2001; Kalinin and Gesser 2002; Overgaard et al. 2005; Gesser 2006). Elevated $[K^+]_0$ depolarizes the membrane potential and shortens the ventricular action potential, which reduces Ca^{2+} influx and contractility (Paterson et al. 1993; Nielsen and Gesser 2001). In painted turtles, an elevation of $[K^+]_0$ from 2.5 to 10 mM reduces twitch force of ventricular strips by 50–95% (Overgaard et al. 2005), and it also results in unstable contractions and lowering of the maximal frequency at which regular contractions may be produced (Nielsen and Gesser 2001; Kalinin and Gesser 2002; Overgaard et al. 2005; Gesser 2006). In fact, hyperkalemia appears to be of major importance as it seems to be the most potent depressor of cardiac force development in cold anoxic turtles. Thus, it is possible that a progressive increase in potassium levels may ultimately compromise sustained cardiac activity in overwintering turtles (Overgaard et al. 2005).

6.3 Regulation of Hypoxic Contractility by Adrenaline and Ca^{2+}

Myocardial contractility can be stimulated by catecholamines and/or elevations of extracellular Ca^{2+}, which increase the intracellular Ca^{2+} transients during the myocardial action potential (e.g., Kitazawa 1984; Bers 2002). For the mammalian myocardium this response seems to be considerably depressed by hypoxia, but the situation may be different for the myocardium of ectothermic vertebrates. Hence the myocardium from both eel and rainbow trout displayed a pronounced increase in isometric force in response to adrenaline under both oxygenated and anoxic conditions (e.g., Gesser et al. 1982). Similarly, an elevation of extracellular Ca^{2+} from 1.25 to 5 mM stimulated force development and glycolysis during anoxia in trout heart muscle (Nielsen and Gesser 1983). Likewise, the combination of adrenaline and elevations of extracellular Ca^{2+} stimulated force development in anoxic myocardial preparations from freshwater turtle (Overgaard et al. 2005). Clearly, there is room for considerable improvements of contractility during anoxia which indicates that anoxia does not necessarily elicit the full anaerobic capacity in the heart muscle of many ectothermic vertebrates.

Adrenergic stimulation increases Ca^{2+} currents through L-type Ca^{2+}-channels in the sarcolemma, and thereby exerts a positive inotropic effect (Bers 2002). Indeed, adrenergic stimulation increases the duration of the action potential in turtles, although this was only significant when the action potential had previously been compromised by hyperkalemia (Nielsen and Gesser 2001), and adrenaline increases twitch force in ventricular strips from *Trachemys* (Ball and Hicks 1996; Nielsen and Gesser 2001; Overgaard et al. 2005; Galli et al. 2006a). These effects are consistent with the rise in stroke volume, which occurs upon adrenergic stimulation in vivo (Hicks and Wang 1998; Overgaard et al. 2002). The positive effects on contractility induced by catecholamines and elevations of extracellular Ca^{2+} are of physiological interest. Hence the spongy myocardium of fish would be expected to receive less oxygen and experience oxygen lack in situations with high

animal activity, which typically involves catecholamine stimulation. Furthermore, the freshwater turtle hibernating with low or no access to oxygen has been shown to have increased levels of both catecholamines (Wasser and Jackson 1991; Keiver et al. 1992) and large increases in extracellular Ca^{2+}. Here Ca^{2+} is released as a result of the progressive buffering by calcium-carbonate stores in the carapace (see Jackson 2002). An increase in plasma Ca^{2+} may also occur in other species (Ruben and Bennett 1981). For both adrenergic stimulation and hypercalcemia, the potential positive effects may be more prominent in alleviating other negative inotropic agents associated with anoxia, tending to reduce sarcolemmal Ca^{2+} influx. Indeed, hypercalcemia and in particular adrenaline have been shown to alleviate negative inotropic effects of hyperkalemia and acidosis in freshwater turtles and several teleost species (Yee and Jackson 1984; Jackson 1987; Nielsen and Gesser 2001; Overgaard et al. 2005; Gesser 2006).

7 Conclusion

The mechanical performance of the heart muscle under hypoxia/anoxia varies largely among species in a way that mostly, but not always, can be related to the environment and behavior of the animal. It is positively correlated to the glycolytic capacity relative to the aerobic capacity and tends to be high in hearts having a low aerobic or total working capacity. Despite the restricted energy production, hypoxic/anoxic performance often responds strongly to positive inotropic agents such as adrenaline. Frequently, mechanical performance appears to be less reduced by the oxygen lack itself than by other factors such as increases in extracellular K^+ commonly associated with hypoxia/anoxia in the living animal. The relation between mechanical performance and the excitation–contraction coupling during hypoxia/anoxia appears ambiguous.

References

Allen, D.G., Morris, P.G., Orchard, C.H. and Pirolo, J.S. (1985) A nuclear magnetic resonance study of metabolism in the ferret heart during hypoxia and inhibition of glycolysis. *J. Physiol*. 361: 185–204.

Andersen, J.B., Gesser, H. and Wang, T. (2004) Acidosis counteracts the negative inotropic effect of K+ on ventricular muscle strips from the toad *Bufo marinus*. *Physiol. Biochem. Zool*. 77: 223–231.

Andrienko, T., Kuznetsov, A.V., Kaambre, T., Usson, Y., Orosco, A., Appaix, F., Tiivel, T., Sikk, P., Vendelin, M., Margreiter, R. and Saks, V.A. (2003) Metabolic consequences of functional complexes of mitochondria, myofibrils and sarcoplasmic reticulum in muscle cells. *J. Exp. Biol*. 206: 2059–2072.

Baker, J.E., Konorev, E.A., Gross, G.J., Chilian, W.M. and Jacob, H.J. (2000) Resistance to myocardial ischemia in five rat strains: is there a genetic component of cardioprotection? *Am. J. Physiol*. 278: H1395–H1400.

Ball, D.C. and Hicks, J.W. (1996) Adrenergic and cholinergic response of ventricular muscle from the turtle. *Trachemys* (*Pseudemys*) *scripta*. *Comp. Biochem. Physiol. A*. 113: 135–141.

Battiprolu, P.K., Harmon, K.J. and Rodnick, K.J. (2007) Sex differences in energy metabolism and performance of teleost cardiac tissue. *Am. J. Physiol*. 292: R827–R836.

Bers, D.M. (2002) Cardiac excitation–contraction coupling. *Nature*. 415: 198–205.

Birkedal, R. and Gesser, H. (2004) Effects of hibernation on mitochondrial regulation and metabolic capacities in myocardium of painted turtle (*Chrysemys picta*). *Comp. Biochem. Physiol. A*. 139: 285–291.

Birkedal, R. and Gesser, H. (2006) Intracellular compartmentation of cardiac fibres from rainbow trout and Atlantic cod — a general design of heart cells. *Biochim. Biophys. Acta*. 1757: 764–772.

Bobb, V.T. and Jackson, D.C. (2005) Effect of graded hypoxic and acidotic stress on contractile force of heart muscle from hypoxia-tolerant and hypoxia-intolerant turtles. *Exp Zool. A*. 303: 345–353.

Boutilier, R.G. (2001) Mechanisms of cell survival in hypoxia and hypothermia. *J. Exp. Biol*. 204: 3171–3181.

Boutilier, R.G. and St-Pierre, J. (2000) Surviving hypoxia without really dying. *Comp. Biochem. Physiol. A*. 126: 481–490.

Bowser, D., Minamikawa, N.T., Nagley, P. and Williams, D.A. (1998) Role of mitochondria in calcium regulation of spontaneously contracting muscle cells. *Biophys. J*. 75: 2004–2014.

Brand, M.D. (2005) The efficiency and plasticity of mitochondrial energy transduction. *Biochem. Soc. Trans*. 33: 897–904.

Buck, L., Espanol, M., Litt, L. and Bickler, P. (1998) Reversible decreases in ATP and PCr concentrations in anoxic turtle brain. *Comp. Biochem. Physiol. A*. 120: 633–639.

Cameron, J.S., Hoffmann, K.E., Zia, C., Hemmett, H.M., Kronsteiner, A. and Lee, C.M. (2003) A role for nitric oxide in hypoxia-induced activation of cardiac KATP channels in goldfish (*Carassius auratus*). *J. Exp. Biol*. 206: 4057–4065.

Chang, J., Knowlton, A.A. and Wasser, J.S. (2000) Expression of heat shock proteins in turtle and mammal hearts: relationship to anoxia tolerance. *Am. J. Physiol*. 278: R209–R214.

Chen, J., Zhu, J.X., Wilson, I. and Cameron, J.S. (2005) Cardioprotective effects of K ATP channel activation during hypoxia in goldfish *Carassius auratus*. *J. Exp. Biol*. 208: 2765–2772.

Christensen, M., Hartmund, T., Gesser, H. (1994) Creatine kinase, energy-rich phosphates and energy metabolism in heart muscle of different vertebrates. *J. Comp. Physiol. B*. 164: 118–123.

Cooke, R. and Pate, E. (1985) The effects of ADP and phosphate on the contraction of muscle fibers. *Biophys. J*. 48: 789–798.

Crampin, E.J. and Smith, N.P. (2006) A dynamic model of excitation–contraction coupling during acidosis in cardiac ventricular myocytes. *Biophys. J*. 90: 3074–3090.

Davie, P.S. and Farrell, A.P. (1991) The coronary and luminal circulation of the fishes. *Can. J. Zool*. 69: 1993–2000.

Driedzic, W.R., Sidell, B.D., Stowe, D. and Branscombe, R. (1987) Matching of vertebrate cardiac energy demand to energy metabolism. *Am. J. Physiol*. 252: R930–R937.

Driedzic, W.R. and Gesser, H. (1994) Energy metabolism and contractility in ectothermic vertebrate hearts: hypoxia, acidosis and low temperature. *Physiol. Rev*. 79: 635–659.

Dröse, S., Brandt, U. and Hanley, P.J. (2006) K^+-independent actions of diazoxide question the role of inner membrane K ATP channels in mitochondrial cytoprotective signaling. *J Biol Chem*. 281: 23733–23739.

Farrar, R.S., Battiprolu, P.K., Pierson, N.S. and Rodnick, K.J. (2006) Steroid-induced cardiac contractility requires exogenous glucose, glycolysis and the sarcoplasmic reticulum in rainbow trout. *J. Exp. Biol*. 209: 2114–2128.

Farrell, A.P. and Clutterham, S.M. (2003) On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. *J. Exp. Biol*. 206: 487–496.

Farrell, A.P. and Stecyk, J.A.W. (2007) The heart as a working model to explore themes and strategies for anoxic survival in ectothermic vertebrates. *Comp. Biochem. Physiol. A*. 147: 300–312.

Faust, H.A., Gamperl, A.K. and Rodnick, K.J. (2004) All rainbow trout (*Oncorhynchus mykiss*) are not created equal: intra-specific variation in cardiac hypoxia tolerance. *J. Exp. Biol*. 207: 1005–1015.

Fukuda, N., O-Uchi, J., Sasaki, D., Kajiwara, H., Ishiwata, S. and Kurihara, S. (2001) Acidosis or inorganic phosphate enhances the length dependence of tension in rat skinned cardiac muscle. *J. Physiol*. 536: 153–160.

Galli, G.L., Gesser, H., Taylor, E.W., Shiels, H.A. and Wang, T. (2006a) The role of the sarcoplasmic reticulum in the generation of high heart rates and blood pressures in reptiles. *J. Exp. Biol*. 209: 1956–1963.

Galli, G.L., Taylor, E.W. and Shiels, H.A. (2006b) Calcium flux in turtle ventricular myocytes. *Am. J. Physiol*. 291: R1781–R1789.

Gesser, H. (1977) The effects of hypoxia and reoxygenation on force development in myocardia of carp and rainbow trout: protective effects of CO_2/HCO_3. *J. Exp. Biol*. 69: 199–206.

Gesser, H. (2002) Mechanical performance and glycolytic requirement in trout ventricular muscle. *J. Exp. Zool*. 293: 360–367.

Gesser, H. (2006) Force development at elevated $[Mg^{2+}]_o$ and $[K^+]_0$ in myocardium from the freshwater turtle (*Trachemys scripta*) and influence of factors associated with hibernation. *Comp. Biochem. Physiol. A*. 145: 334–339.

Gesser, H. and Poupa, O. (1974) Relations between heart muscle enzyme pattern and directly measured tolerance to acute anoxia. *Comp. Biochem. Physiol*. 48: 97–103.

Gesser, H. and Poupa, O. (1978) The role of intracellular Ca^{2+} under hypercapnic acidosis of cardiac muscle: comparative aspects. *J. Comp. Physiol. B*. 127: 301–313.

Gesser, H., Andresen, P., Brams, P. and Sundlarsen, J. (1982) Inotropic effects of adrenaline on the anoxic or hypercapnic myocardium of rainbow trout and eel. *J. Comp. Physiol*. 147: 123–128.

Gesser, H. and Jørgensen, E. (1982) pH_i, contractility and Ca-balance under hypercapnic acidosis in the myocardium of different vertebrate species. *J. Exp. Biol*. 96: 405–412.

Gesser, H. and Poupa, O. (1983) Acidosis and cardiac muscle contractility: comparative aspects. *Comp. Biochem. Physiol A*. 76: 559–566.

Gibbs, C.L. (2003) Cardiac energetics: sense and nonsense. *Clin. Exp. Pharmacol. Physiol*. 30: 598–603.

Gnaiger, E. (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir. Physiol*. 128: 277–297.

Godt, R.E. and Nosek, T.M. (1989) Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. *J. Physiol*. 412: 155–180.

Hansen, C.A. and Sidell, B.D. (1983) Atlantic hagfish cardiac muscle: metabolic basis of tolerance to anoxia. *Am. J. Physiol*. 244: R356–R362.

Hardie, D.G. (2000) Metabolic control: a new solution to an old problem. *Curr. Biol*. 10: R757–R759.

Hartmund, T. and Gesser, H. (1996) Cardiac force and high-energy phosphates under metabolic inhibition in four ectothermic vertebrates. *Am. J. Physiol*. 271: R946–R954.

Hicks, J.W. and Wang, T. (1998) Cardiovascular regulation during anoxia in the turtle: an *in vivo* study. *Physiol. Zool*. 71: 1–14.

Hochachka, P.W. (1986) Defense strategies against hypoxia and hypothermia. *Science* 231: 234–241.

Hochachka, P.W., Buck, L.T., Doll, C.J. and Land, S.C. (1996) Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. *Proc. Nat. Acad. Sci. U. S. A*. 93: 9493–9498.

Holmuhamedov, E.L., Jovanovic, S., Dzeja, P.P., Jovanovic, A. and Terzic, A. (1998) Mitochondrial ATP-sensitive K^+ channels modulate cardiac mitochondrial function. *Am. J. Physiol*. 275: H1567–H1576.

Hove-Madsen, L., Llach, A., Tibbits, G.F. and Tort, L. (2003) Triggering of sarcoplasmic reticulum Ca^{2+} release and contraction by reverse mode Na^+/Ca^{2+} exchange in trout atrial myocytes. *Am. J. Physiol*. 284: R1330–R1339.

Jackson, D.C. (1968) Metabolic depression and oxygen depletion in diving turtle. *J. Appl. Physiol*. 24: 503–509.

Jackson, D.C. (1987) Cardiovascular function in turtles during anoxia and acidosis: *in vivo* and *in vitro* studies. *Amer. Zool*. 27: 49–58.

Jackson, D.C. (2000) Living without oxygen: lessons from the freshwater turtle. *Comp. Biochem. Physiol. A*. 125: 299–315.

Jackson, D.C. (2002) Hibernating without oxygen: the painted turtle. *J. Physiol*. 543: 731–737.

Jackson, D.C. and Heisler, N. (1982) Plasma ion balance of submerged anoxic turtles at 3°C. *Respir. Physiol*. 49: 159–174.

Jackson, D.C. and Ultsch, G.R. (1982) Long-term submergence at 3°C of the turtle *Chrysemys picta bellii*, in normoxic and severely hypoxic water: II. Extracellular ionic responses to extreme lactic acidosis. *J. Exp. Biol*. 96: 29–43.

Jackson, D.C., Shi, H., Singer, J.H., Hamm, P.H. and Lawler, R.G. (1995) Effects of input pressure on *in vitro* turtle heart during anoxia and acidosis: a 31P-NMR study. *Am. J. Physiol*. 268: R683–R689.

Jarmakani, J.M., Nakazawa, M., Nagatomo, T. and Langer, G.A. (1978) Effect of hypoxia on mechanical function in the neonatal mammalian heart. *Am. J. Physiol*. 235: H469–H474.'

Jensen, M. and Gesser, H. (1999) Influence of inorganic phosphate and energy state on force in skinned cardiac muscle from freshwater turtle and rainbow trout. *J. Comp Physiol*. 169: 439–444.

Joseph, T., Coirault, C. and Lecarpantier, Y. (2000) Species-dependent changes in mechano-energetics of isolated cardiac muscle during hypoxia. *Basic Res. Cardiol*. 95: 378–384.

Kalinin, A. and Gesser, H. (2002) Oxygen consumption and force development in turtle and trout cardiac muscle during acidosis and high extracellular potassium. *J. Comp. Physiol. B*. 172: 145–151.

Kammermeier, H. (1997) Myocardial cell energetics. *Adv. Exp. Med. Biol*. 430: 89–96.

Keiver, K.M., Weinberg, J. and Hochachka, P.W. (1992) Roles of catecholamines and corticosterone during anoxia and recovery at 5°C in turtle. *Am. J. Physiol*. 263: R770–R774.

Kitazawa, T. (1984) Effect of extracellular calcium on contractile activation in guinea-pig ventricular muscle. *J. Physiol*. 355: 635–659.

Kockskamper, J., Zima, A.V. and Blatter, L.A. (2005) Modulation of sarcoplasmic reticulum Ca2+ release by glycolysis in cat atrial myocytes. *J. Physiol*. 564: 697–714.

Lehninger, A.L. (1970) Biochemistry. Worth, New York

Lorenz, J.N. and Paul, R.J. (1997) Dependence of Ca^{2+} channel currents on endogenous and exogenous sources of ATP in portal vein smooth muscle. *Am. J. Physiol*. 272: H987–H994.

Lutz, P.L. and Nilsson, G.E. (1997) Contrasting strategies for anoxic brain survival — glycolysis up or down. *J. Exp. Biol*. 200: 411–419.

MacCormack, T.J. and Driedzic, W.R. (2002) Mitochondrial ATP-sensitive K^+ channels influence force development and anoxic contractility in a flatfish, yellowtail flounder *Limanda ferruginea*, but not Atlantic cod *Gadus morhua* heart. *J. Exp. Biol*. 205: 1411–1418.

MacCormack, T.J. and Driedzic, W.R. (2007) The impact of hypoxia on in vivo glucose uptake in a hypoglycemic fish, *Myoxocephalus scorpius*. *Am. J. Physiol*. 292: R1033–R1042.

Meyer, R.A. (1988) A linear model of muscle respiration explains monoexponential phosphocreatine changes. *Am. J. Physiol*. 254: C548–C533.

Mühlfeld, C., Singer, D., Engelhardt, N., Richter, J. and Schmiedl, A. (2005) Electron microscopy and microcalorimetry of the postnatal rat heart (*rattus norvegicus*). *Comp. Biochem. Physiol. A*. 141: 310–318.

Nielsen, J.S. and Gesser, H. (2001) Effects of high extracellular $[K^+]$ and adrenaline on force development, relaxation and membrane potential in cardiac muscle from freshwater turtle and rainbow trout. *J. Exp. Biol*. 204: 261–268.

Nielsen, K.E. and Gesser, H. (1983) Effects of $[Ca^{2+}]_O$ on contractility in the anoxic cardiac-muscle of mammal and fish. *Life Sci*. 32: 1437–1442.

Nilsson, G.E. and Renshaw, G.M. (2004) Hypoxic survival strategies in two fishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. *J. Exp. Biol*. 207: 3131–3139.

Noma, A. (1983) ATP-regulated K^+ channels in cardiac muscle. *Nature* 305: 147–148.

Ostadal, B., Ostadalova, I. and Dhalla, N.S. (1999) Development of cardiac sensitivity to oxygen deficiency: comparative and ontogenic aspects. *Physiol. Rev*. 74: 221–258.

Orchard, C.H., and Kentish, J.C. (1990) Effects of changes of pH on the contractile function of cardiac muscle. *Am. J. Physiol*. 258: C967–C981.

Overgaard, J., Stecyk, J.A.W., Farrell, A.P. and Wang, T. (2002) Adrenergic control of the cardiovascular system in the turtle *Trachemys scripta. J. Exp. Biol*. 205: 3335–3345.

Overgaard, J. and Gesser, H. (2004) Force development, energy state and ATP production of cardiac muscle from turtles and trout during normoxia and severe hypoxia. *J. Exp. Biol*. 207: 1915–1924.

Overgaard, J., Wang, T., Nielsen, O.B. and Gesser, H. (2005) Extracellular determinants of cardiac contractility in the cold, anoxic turtle. *Physiol. Biochem. Zool*. 78: 976–995.

Overgaard, J., Gesser, H. and Wang, T. (2007) Cardiac performance and cardiovascular regulation during anoxia/hypoxia in freshwater turtles. *J. Exp. Biol*. 210: 1687–1699.

Paajanen, V. and Vornanen, M. (2003) Effects of chronic hypoxia on inward rectifier K^+ current (I(K1)) in ventricular myocytes of crucian carp (*Carassius carassius*) heart. *J. Membr. Biol*. 194: 119–127.

Paterson, D.J., Rogers, J., Powell, T. and Brown, H.F. (1993) Effect of catecholamines on the ventricular myocyte action-potential in raised extracellular potassium. *Acta Physiol. Scand*. 148: 177–186.

Perry, S. and Reid, S. (1994) The effects of acclimation temperature on the dynamics of catecholamine release during acute hypoxia in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol*. 186: 289–307.

Poupa, O., Gesser, H. and Johansen, K. (1978) Myocardial inotrophy of CO2 in water- and air-breathing vertebrates. *Am J Physiol*. 234: R155–R157.

Reese, S.A., Jackson, D.C. and Ultsch, G.R. (2002) The physiology of overwintering in a turtle that occupies multiple habitats, the common snapping turtle (*Chelydra serpentina*). *Physiol. Biochem. Zool*. 75: 432–438.

Reeves, R.B. (1963) Control of glycogen utilization and glucose uptake in the anaerobic turtle heart. *Am. J. Physiol*. 205: 23–29.

Ruben, J.A. and Bennett, A.F. (1981) Intense exercise, bone structure and blood calcium levels in vertebrates. *Nature*. 291: 411–413.

Saks, V., Dzeja, P., Schlattner, U., Vendelin, M., Terzic, A. and Wallimann, T. (2006) Cardiac system bioenergetics: metabolic basis of the Frank–Starling law. *J. Physiol*. 571: 253–273.

Shi, H. and Jackson, D.C. (1997) Effects of anoxia, acidosis and temperature on the contractile properties of turtle cardiac muscle strips. *J. Exp. Biol*. 200: 1965–1973.

Shi, H., Hamm, P.H., Lawler, R.G. and Jackson, D.C. (1999) Different effects of simple anoxic lactic acidosis and simulated *in vivo* anoxic acidosis on turtle heart. *Comp. Biochem. Physiol. A*. 122: 173–180.

Stecyk, J.A., Paajanen, V., Farrell, A.P., Vornanen, M. (2007) Effect of temperature and prolonged anoxia exposure on electrophysiological properties of the turtle (*Trachemys scripta*) heart. *Am. J. Physiol*. 293: R421–R437.

Storey, K.B. and Storey, J.M. (1990) Facultative metabolic rate depression in animals: molecular regulation and biochemical adaptation in anaerobiosis, hibernation, and estivation. *Q. Rev. Biol*. 65: 145–174.

Teague, W.E. Jr., Golding, E.M. and Dobson, G.P. (1996) Adjustment of K' for the creatine kinase, adenylate kinase and ATP hydrolysis equilibria to varying temperature and ionic strength. *J. Exp. Biol*. 199: 509–512.

Thomas, M.J., Hamman, B.N. and Tibbits, G.F. (1996) Dihydropyridine and ryanodine binding in ventricles from rat, trout, dogfish and hagfish. *J. Exp. Biol*. 199: 1999–2009.

Tibbits, G.F., Kashihara, H., Thomas, M.J., Keen, J.E. and Farrell, A.P. (1990) Ca^{2+} transport in myocardial sarcolemma from rainbow trout. *Am. J. Physiol*. 259: R453–R460.

Tota, B. (1983) Vascular and metabolic zonation in the ventricular myocardium of mammals and fishes. *Comp. Biochem. Physiol. A*. 76: 423–438.

Ultsch, G.R. and Jackson, D.C. (1982) Long-term submergence at 3 °C of the turtle, *Chrysemys picta bellii*, in normoxic and severely hypoxic water. I. Survival, gas exchange and acid-base status. *J. Exp. Biol*. 96: 11–28.

Ultsch, G.R., Carwile, M.E., Crocker, C.E. and Jackson, D.C. (1999) The physiology of hibernation among painted turtles: the eastern painted turtle (*Chrysemys picta picta*). *Physiol. Biochem. Zool*. 72: 493–501.

van den Thillart, G., van Waarde, A., Muller, H.J., Erkelens, C., Addink, A. and Lugtenburg, J. (1989) Fish muscle energy metabolism measured by in vivo 31P-NMR during anoxia and recovery. *Am. J. Physiol*. 256: R922–R929.

van Warde, A. (1991) Alcoholic fermentation in multicellular organisms. *Physiol. Zool*. 64: 895–920.

Vleugels, A., Carmeliet, E., Bosteels, S. and Zaman, M. (1976) Differential effects of hypoxia with age on the chick embryonic heart. Changes in membrane potential, intracellular K and Na, K efflux and glycogen. *Pflügers Arch*. 30: 159–166.

Vornanen, M. (1999) Na^{+}/Ca^{2+} exchange current in ventricular myocytes of fish heart: contribution to sarcolemmal Ca^{2+} influx. *J. Exp. Biol*. 202: 1763–1775.

Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K., Eppenberger, H.M. (1992) Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. *Biochem. J*. 281: 21–40.

Wasser, J.S., Inman, K.C., Arendt, E.A., Lawler, R.G. and Jackson, D.C. (1990) 31P-NMR measurements of pH_i and high-energy phosphates in isolated turtle hearts during anoxia and acidosis. *Am. J. Physiol*. 28: R521–R530.

Wasser, J.S. and Jackson, D.C. (1991) Effects of anoxia and graded acidosis on the levels of circulating catecholamines in turtles. *Respir. Physiol*. 84: 363–377.

Westerblad, H., Allen, D.G. and Lännergren, J. (2002) Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol. Sci*. 17: 17–21.

Yee, H.F., Jackson, D.C. (1984) The effects of different types of acidosis and extracellular calcium on the mechanical activity of turtle atria. *J. Comp. Physiol*. 154: 385–391.

Yellon, D.M., Baxter, G.F., Garcia-Dorado, D., Heusch, G. and Sumeray, M.S. (1998) Ischaemic preconditioning: present position and future directions. *Cardiovasc. Res*. 37: 21–33.

Zaar, M., Overgaard, J., Gesser, H. and Wang, T. (2007) Contractile properties of the functionally divided python heart: two sides of the same matter. *Comp. Biochem. Physiol. A*. 146: 163–173.