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ORIGINAL PAPER

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Responses of the red blood cells from two high-energy-demand teleosts, yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*), to catecholamines

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Abstract In fishes, catecholamines increase red blood cell intracellular pH through stimulation of a sodium/proton (Na^+/H^+) antiporter. This response can counteract potential reductions in blood O_2 carrying capacity (due to Bohr and Root effects) when plasma pH and intracellular pH decrease during hypoxia, hypercapnia, or following exhaustive exercise. Tuna physiology and behavior dictate exceptionally high rates of O_2 delivery to the tissues often under adverse conditions, but especially during recovery from exhaustive exercise when plasma pH may be reduced by as much as 0.4 pH units. We hypothesize that blood O_2 transport during periods of metabolic acidosis could be especially critical in tunas and the response of rbc to catecholamines elevated to an extreme. We therefore investigated the in vitro response of red blood cells from yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*) to catecholamines. Tuna red blood cells had a typical response to catecholamines, indicated by a rapid decrease in plasma pH. Amiloride reduced the response, whereas 4,4′diisothiocyanatostilbene-2,2′-disulphonic acid enhanced both the decrease in plasma pH and the increase in intracellular pH. Changes in plasma [Na^+], [Cl^-], and [K^+] were consistent with the hypothesis that tuna red blood cells have a Na^+/H^+ antiporter similar to that described for other teleost red blood cells. Red blood cells from both tuna species were more responsive to noradrenaline than adrenaline. At identical catecholamine concentrations, the decrease in plasma pH was greater in skipjack tuna blood, the more active of the two tuna species. Based on changes in plasma pH, the response of red blood cells to catecholamines from both tuna species was less than that of rainbow trout (*Oncorhynchus mykiss*) red blood cells, but greater than that of cod (*Gadus morhua*) red blood cells. Noradrenaline had no measurable influence on the O_2 affinity of skipjack tuna blood and only slightly increased the O_2 affinity of yellowfin tuna blood. Our results, therefore, do not support our original hypothesis. The catecholamine response of red blood cells from high-energy-demand teleosts (i.e., tunas) is not enhanced compared to other teleosts. There are data on changes in cardio-respiratory function in tunas caused by acute hypoxia and modest increases in activity, but there are no data on the changes in cardio-respiratory function in tunas accompanying the large increases in metabolic rate seen during recovery from exhaustive exercise. However, we conclude that during those instances where high rates of O_2 delivery to the tissues are needed, tunas' ability to increase cardiac output, ventilation volume, blood O_2 carrying capacity, and effective respiratory (i.e., gill) surface area are probably more important than are the responses of red blood cells to catecholamines. We also use our data to investigate the extent of the Haldane effect and its relationship to blood O_2 and CO_2 transport in yellowfin tuna. Yellowfin tuna blood shows a large Haldane effect; intracellular pH increases 0.20 units during oxygenation. The largest change in intracellular pH occurs between 40–100% O_2 saturation, indicating that yellowfin tuna, like other teleosts, fully exploit the Haldane effect over the normal physiological range of blood O_2 saturation.

Key words Adrenaline · Noradrenaline · Na^+/H^+ antiporter · Oxygen · RBC

Abbreviations *DIDS* 4,4′ diisothiocyanatostilbene-2,2′-disulphonic acid · EC_{50} drug concentrations producing a response 50% of the maximal response · *Hb* hemoglobin · P_{50} pO_2 required to reach 50% O_2 saturation · pH_{50} blood pH at P_{50} · pH_e true plasma pH · pH_i red blood cell

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intracellular pH · pO_2 partial pressure of oxygen · pCO_2 partial pressure of carbon dioxide · $[O_2]$ blood oxygen content · *RBC* red blood cells

Introduction

Catecholamines are released into the circulatory system of teleosts during situations requiring enhanced blood O_2 transport; exhaustive exercise, hypercapnia, and hypoxia are typical stimuli (Thomas and Perry 1992). Following exhaustive exercise, protons released from muscle generate a metabolic acidosis (i.e., reductions in plasma pH, pH_e). This, in turn, can cause a decrease in blood O_2 affinity (due to Bohr and Root effects) which persists at the gills (Wood and Perry 1985). During these periods, circulating catecholamines preserve blood O_2 transport by increasing or maintaining red blood cell (RBC) internal pH (pH_i) by stimulation of a sodium/proton (Na^+/H^+) antiporter (Cossins and Richardson 1985; Nikinmaa 1986; Tetens et al. 1988; Tufts and Randall 1989; Jensen 1991; Nikinmaa 1997). Indeed, lowered arterial O_2 content appears the likely stimulus for the release of catecholamines in fish (Nikinmaa 1992; Thomas and Perry 1992; Perry and Gilmour 1996).

The influence of catecholamines on pH_e, pH_i, and blood O_2 transport has been extensively studied in salmonids (Nikinmaa 1983; Cossins and Richardson 1985; Boutilier et al. 1986; Primmett et al. 1986; Steffensen et al. 1987; Perry and Kinkead 1989; Tufts et al. 1991; Thomas and Perry 1992) and a few other marine and freshwater teleosts (Milligan and Wood 1987; Salama and Nikinmaa 1988, 1989; Berenbrink and Bridges 1994; Roig et al. 1997). The available data imply that the magnitude of the RBC response to catecholamines is linked to activity patterns and is affected by pH_e, and blood partial pressures of O_2 and CO_2 (pO_2 and pCO_2, respectively). RBC from active teleosts, such as rainbow trout (*Oncorhynchus mykiss*), are responsive under normoxia but hypoxia and low pH_e accentuate the response (Borgese et al. 1987; Cossins and Kilbey 1989). RBC from sluggish species, such as tench (*Tinca tinca*) and carp (*Cyprinus carpio*), are largely insensitive to catecholamines during normoxia but display increased sensitivity during acute hypoxia and decreased pH_e (Jensen 1987; Nikinmaa et al. 1987; Salama and Nikinmaa 1988). However, the response of RBC from high-energy-demand pelagic species (i.e., tunas) to catecholamines have not been measured.

Tunas (family *Scombridae*, tribe *Thunnini*) are one of the most active groups of fish and have standard and active metabolic rates several times higher than those of other teleosts (Bushnell and Jones 1994; Brill 1996). High O_2 delivery rates by the cardiovascular and respiratory systems are due to a suite of biochemical, anatomical, and physiological adaptations (Brill and Bushnell 1991b; Bushnell and Brill 1992; Bushnell and Jones 1994; Dickson 1996; Korsmeyer et al. 1996a). Tunas are also obligate ram ventilators and must swim continuously. During their normal daily vertical movements, they routinely subject themselves to hypoxic water and rapid ambient temperature changes (Dizon et al. 1979; Sund et al. 1981; Holland et al. 1990; Cayre and Marsac 1993; Brill 1994; Block et al. 1997). Thus, tuna physiology and behavior dictate high rates of O_2 delivery to the tissues often under adverse conditions.

Moreover, during exhaustive exercise, tunas can generate some of the highest white muscle lactate levels of any vertebrate (up to 150 $\mu M \cdot g^{-1}$: Guppy et al. 1979; Arthur et al. 1992). Tunas can also clear white muscle lactate at rates about 20 times higher than those seen in salmonids; rates that are comparable to those of mammals even when muscle temperatures in tunas are only 25 °C (Barrett and Connor 1964; Arthur et al. 1992). Such high rates of lactate clearance require tunas to increase their already high routine O_2 delivery to the tissues ($\approx$250–350 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) by as much as 5–10 times (Gooding et al. 1981; Bushnell and Jones 1994; Dewar and Graham 1994; Korsmeyer et al. 1996a, 1997b), but at a time when arterial blood pH may be reduced by as much as 0.4 pH units (Perry et al. 1985; Brill et al. 1992). Therefore, we hypothesize that the response of tuna RBC to catecholamines should be elevated, compared to other teleosts, to ensure adequate O_2 delivery during recovery from exhaustive exercise.

To test our hypothesis, we evaluated the responsiveness of RBC from yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*) to catecholamines by constructing dose-response curves. Responsiveness was quantified by measuring reductions in pH_e following exposure to adrenaline and noradrenaline. Using selective blockers – amiloride and 4,4′ diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS) – we also investigated the mechanisms of H^+ extrusion in yellowfin tuna RBC. In addition, we quantified the effect of noradrenaline on yellowfin and skipjack tuna blood O_2 affinity.

We also use our data to investigate the extent of the Haldane effect and its relationship to blood O_2 and CO_2 transport in yellowfin tuna. The hemoglobin (Hb) from different species of fish shows major interspecific differences in functional properties with respect to O_2 and CO_2 transport (Jensen 1991). These differences are reflected in the Bohr and Haldane effects. In brief, the Bohr effect is thought to primarily augment O_2 unloading at the tissue while the Haldane effect benefits CO_2 transport (Jensen 1991).

Materials and methods

Fish maintenance, handling, and surgical procedures

Yellowfin and skipjack tunas were purchased from local commercial fishermen and maintained in outdoor tanks (water temperature 25 ± 2 °C) at the Kewalo Research Facility (National Marine Fisheries Service, Southwest Fisheries Science Center, Honolulu Laboratory). Fish were held for a few days to several weeks before use. Food was presented daily, but not for approximately 20 h prior to use in an experiment to allow sufficient time for gut

clearance (Magnuson 1969). We used a total of 20 yellowfin tuna and 16 skipjack tuna (approximate body weights 1–2 kg).

To obtain blood with normal acid-base status and lactate levels, we followed procedures described in Brill and Bushnell (1991a). Briefly, to achieve initial anesthesia, fish were dip-netted from their holding tank and placed in a plastic bag containing 5 l of oxygenated seawater with 1 g l^{-1} MS222 (Argent Chemical Laboratory, Redmond, Washington) buffered with an equal molar concentration of sodium bicarbonate. Fish were then moved into the laboratory and placed ventral side up on a surgical table. Anesthesia was maintained by pumping seawater over the gills containing 0.1 g l^{-1} MS222, also buffered with an equal molar concentration of sodium bicarbonate. A 20 gauge, 3.2 cm Instyle Vialon I.V. catheter (Becton Dickinson Vascular Access, Sandy, Utah) was introduced into the ventral aorta under manometric guide and connected to a 20 cm length of polyethylene tubing (PE 160). The catheter hub was sutured to the skin immediately anterior to the pelvic fins. Fish were then turned upright, spinally blocked with 2% lidocaine, and placed in front of a pipe delivering oxygenated seawater at approximately 35 $l \cdot min^{-1}$. Under these conditions, the cranial nerves are left intact and cardio-respiratory function is normal (Bushnell et al. 1990; Bushnell and Brill 1991). Fish were allowed to recover until pH_e reached approximately 7.5 or higher (generally 1–2 h). During this period the fish were kept sedated with repeated intramuscular doses (0.1–0.3 ml) of the steroid anesthetic Saffan (Glaxovet, Harefield, Uxbridge, England), which is highly effective in fish (Oswald 1978). Following this, 30–40 ml of blood was withdrawn as quickly as possible but with minimal disturbance. Fish were then immediately killed with an overdose of sodium pentobarbital. To prevent clotting, sodium heparin (10 000 IU ml^{-1}) was added to the blood to final nominal concentration of 100 IU ml^{-1} blood.

To degrade catecholamines released during anesthesia, surgery, recovery, or blood withdrawal, the blood was exposed to bright light for 1 h at room temperature (≈22–25 °C) while gently swirled (Gilmour et al. 1994). The blood was then stored overnight at 4 °C to minimize any residual effects of endogenous catecholamines (Bourne and Cossins 1982; Gallardo Romero et al. 1996; Kaloyianni and Rasidaki 1996). It was warmed to room temperature over 1–2 h while gently swirled before use in any experiment

Catecholamine dose-response curves and mechanisms of H^+ extrusion

For catecholamine dose-response experiments, 1.5 ml blood samples were placed in 50 ml glass tonometers that had been completely covered with black tape to exclude light. The tonometers were maintained in a 25 °C water bath, gently swirled, and continuously flushed with water-vapor-saturated air containing 1% CO_2. This level of CO_2 was chosen because it approximates the pCO_2 of tuna blood in vivo (Jones et al. 1986; Bushnell and Brill 1992). The tonometers were flushed with gas for at least 1 h before the addition of blood. The gas mixture was produced using two MKS Instruments (Andover, Mass.) Mass Flow Controllers (Model 1159B) connected to a MKS digital readout (Model 247C), and stored in an automobile tire inner tube. The pCO_2 of the gas was continuously checked during mixing with a Hewlett-Packard 47210A Capnometer (Hewlett-Packard Germany, D-7030, Böblingen, Germany). The pH of whole blood samples (i.e., pH_e) was determined using standard procedures with a Radiometer MKS Mark 2 blood gas analyzer (Radiometer America, Cleveland, Ohio) maintained at 25 °C. Noradrenaline and adrenaline solutions (norepinephrine bitartrate and epinephrine bitartrate salt, respectively; Sigma Chemical, St Louis, Missouri) were prepared fresh daily in 1.17% NaCl and stored at 4 °C in the dark.

Adrenaline or noradrenaline (100 µl volume) was added to the tonometers in a random order to yield final nominal concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-10} M. Changes in pH_e were measured after 5 min. The time course of the response was determined in yellowfin tuna blood by measuring the change in pH_e at 2.5, 5, 10, 20 and 30 min following addition of either noradrenaline (final nominal concentration of 10^{-5} M) or a saline control.

The ion transport inhibitors DIDS (Sigma Chemical) and amiloride (amiloride hydrochloride; hydrate; Sigma Chemical) were used to investigate the mechanism of H^+ extrusion in yellowfin tuna RBC. Blood samples from an individual fish were placed in six tonometers and treatments applied in duplicate. Noradrenaline (10^{-5} M final concentration) and DIDS (10^{-4} M final concentration) were dissolved in saline, amiloride was dissolved in 1% dimethylsulphoxide (10^{-4} M final concentration). Amiloride was added 20 min and DIDS 10 min prior to the addition of noradrenaline. Control tonometers had only saline added. Samples were taken for pH_e, pH_i, RBC water content, and plasma [Na^+], [Cl^-], and [K^+] prior to and 5 min after the addition of noradrenaline. The pH_i was determined using the freeze/thaw technique to produce a hemolysate from packed RBC (Zeidler and Kim 1977; Nikinmaa 1983). Plasma bicarbonate concentrations were determined using the Cameron method (Cameron 1971). Cell water content was determined by drying a known amount of packed RBC to constant weight. Corrections were not made for trapped extracellular fluid. Plasma [Na^+], [Cl^-], and [K^+] were determined using a Beckman Synchron clinical system CX3 Delta (Beckman Instruments, Brea, Calif.).

The Hb concentrations ([Hb]) of whole blood were determined using the cyanmethemoglobin method (Dacie and Lewis 1984). Changes in pH_e following the addition of catecholamines were corrected for differences in [Hb] as described by Thomas et al. (1991), and all results are presented on the basis of 10 g Hb · dl $blood^{-1}$. The noradrenaline concentrations producing a response 50% of the maximal recorded response (EC_{50}) were determined from Hill plots (5–95% response range) (Tetens et al. 1988). Analysis of covariance was used to determine differences in EC_{50}. The mean pH_e responses to saturating doses of noradrenaline were compared using Student's *t*-test. The effect of ion inhibitors on pH_e, pH_i, cell swelling and extracellular [Na^+], [K^+] and [Cl^-], was determined using either one-way repeated measures analysis of variance or Student's *t*-test. Student's *t*-test was used when the power for the analysis of variance was below 0.8. Under this condition, comparisons were restricted to before and after the addition of noradrenaline for either the control, amiloride, or DIDS treatments.

Effects of catecholamines on blood O_2 binding

Blood from yellowfin and skipjack tuna was obtained and handled as described above. Samples (1.5 ml) were placed in six 50-ml glass tonometers that had been completely covered with black tape to exclude light. The tonometers were flushed for 1 h with water-vapor-saturated gas mixtures of six nominal pO_2 (5, 10, 20, 40, 80 and 150 mm Hg) and 1% CO_2 prior to the introduction of blood samples. To ensure accuracy, gas mixtures were made daily using the gas mixing system described above and stored in automobile tire inner tubes.

Blood O_2 dissociation curves were constructed by measuring both the pO_2 and O_2 content ([O_2]) of sub-samples removed from the tonometers. The former was measured using a Radiometer MKS Mark 2 blood gas analyzer maintained at 25 °C, and the latter as described by Tucker (1967). The pH_e was also measured in samples of both skipjack and yellowfin tuna blood, and pH_i in yellowfin tuna packed RBC as described above. [Hb] and plasma [HCO_3^-] were also quantified as described above.

Two blood O_2 dissociation curves were acquired from the blood taken from each fish. The first was a control, with blood samples taken from the tonometers after 1 h of equilibration. The second was obtained from samples taken 30 min after the addition of noradrenaline (100 µl volume, final nominal concentration in the tonometer 10^{-5} M). This concentration produces maximum cell swelling and decreases in pH_e in both skipjack and yellowfin tuna blood in vitro. It also approximates maximum noradrenaline levels recorded in vivo in restrained rested tunas (Keen et al. 1995).

Blood O_2 dissociation curves were constructed by fitting the data to the logistic function using a least squares regression (Sigmaplot, SPSS, Chicago, Ill.). The P_{50} was calculated using two methods as described in Nikinmaa (1990). In the first method, P_{50} was the pO_2 required to reach 50% blood O_2 saturation, with 100% saturation

taken as the $[O_2]$ measured in blood equilibrated at a pO_2 of approximately 150 mm Hg. In the second method, 100% saturation was the theoretical maximum $[O_2]$ based on measured [Hb], and assuming a Hb O_2 carrying capacity of 1.25 ml $O_2 \cdot g^{-1}$ Hb at 25 °C (Selkurt 1976). The former method takes the Root effect's influence on P_{50} into account, whereas the latter method does not (Nikinmaa 1990). Blood pH at P_{50} (pH_{50}) was determined by regressing pH_e against log pO_2, and then interpolating.

In order to allow direct comparison of Bohr effect coefficients from our study with those previously published on tunas and other teleosts, the Bohr effect coefficient was calculated as $\Delta \log P_{50}/\Delta pH_e$, with P_{50} calculated by both methods described above. The Root effect was calculated from the difference in $[O_2]$ when the blood was equilibrated at a pO_2 of approximately 150 mm Hg and the theoretical maximum blood $[O_2]$ based on [Hb]. Hill numbers were derived by calculating the slope of the linear portion of the regression of log pO_2 versus log [% saturation/(100-% saturation)], with the $[O_2]$ at 100% blood O_2 saturation based on the theoretical maximum $[O_2]$ derived from measured [Hb].

All results in the text, tables and graphs are presented as mean ± SEM. Significant differences in P_{50}, pH_{50}, and pH_i before and after the addition of noradrenaline were tested using paired Student's *t*-test. Significant differences in the Bohr effect coefficient and Root effect before and after the addition of noradrenaline were tested using analysis of covariance. Difference were considered significant when $P < 0.05$ for all statistical tests.

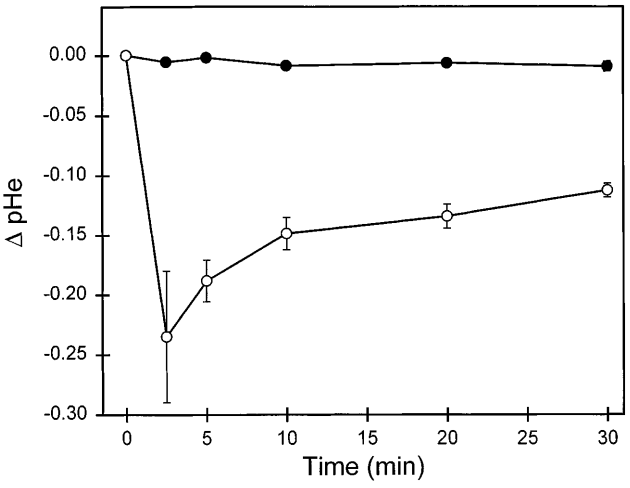

Fig. 1 Change in true plasma pH (pH_e) following the addition of saline (*filled circle*) or noradrenaline (*open circle*, 10^{-5} M final concentration) in yellowfin tuna blood equilibrated to air + 1% CO_2. Values are mean ± SEM ($n = 5$). The absence of *vertical bars* indicates that the SEM falls within the diameter of the circles

Results

Catecholamine dose-response and mechanisms of H^+ extrusion

The hematocrit (35% ± 4% and 35% ± 6%), [Hb] (12 ± 0.5 and 13 ± 0.6 $g \cdot dl^{-1}$), and mean cell Hb concentration (36 ± 0.7 and 37 ± 0.8 $g \cdot dl^{-1}$) (for skipjack and yellowfin tuna, respectively) of the blood samples used in our study all agree with values currently thought to be normal for tunas (Jones et al. 1986; Brill and Bushnell 1991a; Brill and Jones 1994). We found that even slight hemolysis caused significant reductions in pH_e and increases in extracellular $[K^+]$. Although almost never a problem with yellowfin tuna RBC, skipjack tuna RBC appeared more fragile and would occasionally undergo some hemolysis when left in the tonometers for more than 1 h, or during centrifugation to obtain plasma samples. We therefore choose to use skipjack tuna blood only for those experiments requiring minimal time in the tonometers or no centrifugation. Consequently, data on pH_i and the effects of amiloride and DIDS were obtained only from yellowfin tuna RBC.

Noradrenaline caused an immediate decrease in pH_e, with the largest decrease occurring after approximately 2.5 min in yellowfin tuna blood (Fig. 1). Both adrenaline and noradrenaline caused dose-dependent reductions in pH_e in blood from both tuna species (Fig. 2), but RBC were most sensitive to noradrenaline. For noradrenaline, the EC_{50} values were 8.9×10^{-8} M and 4.8×10^{-8} M in yellowfin tuna and skipjack tuna blood, respectively. For adrenaline, the EC_{50} values were approximately an order of magnitude higher, 9.6×10^{-7} M and 7.0×10^{-7} M in yellowfin and skipjack tuna blood, respectively. The slopes of the Hill plots for noradrenaline (Fig. 2, inserts) were not significantly different between

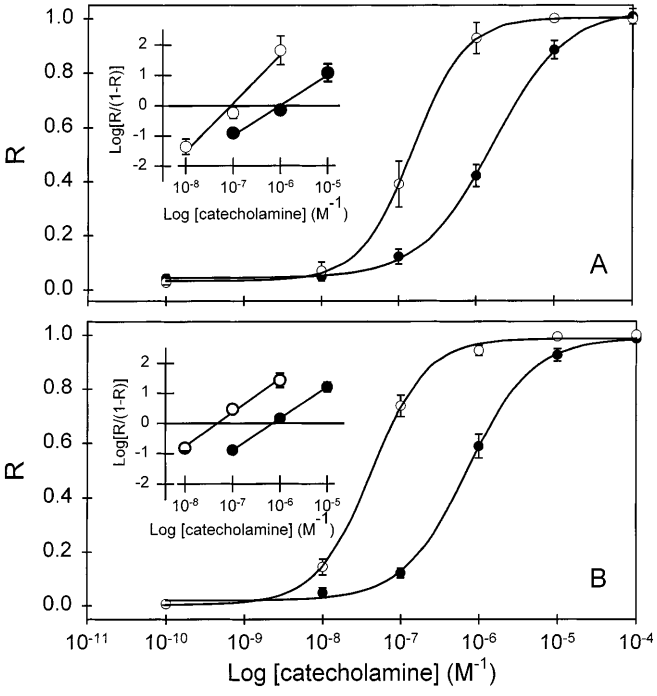

Fig. 2 Dose response curves for yellowfin tuna **A** and skipjack tuna **B** red blood cells following the addition of adrenaline (*filled circle*) and noradrenaline (*open circle*). The response (R) is calculated as a percentage based on the maximum change in pH_e measured 5 min after the addition of catecholamines. Catecholamine concentrations shown are the nominal final concentrations. Hill plots of the dose response curves, based on samples taken 5 min after the addition of adrenaline (*filled circle*) and noradrenaline (*open circle*), are shown as inserts . Values are mean ± SEM ($n = 5$). Equations for the Hill plot regression lines are: Yellowfin tuna: log $[R/(1-R)] = 1.589$ log [noradrenaline] + 11.20, $r = 0.986$, and log $[R/(1-R)] = 0.991$ log [adrenaline]−5.96028, $r = 0.991$; Skipjack tuna: log $[R/(1-R)] = 1.120$ log [noradrenaline] + 8.2025, $r = 0.996$, and log $[R/(1-R)] = 1.048$ log [adrenaline] + 6.449, $r = 0.999$

the two species, whereas those of the EC_{50} plots were. The lower EC_{50} shows skipjack tuna RBC are approximately twice as sensitive to noradrenaline as yellowfin tuna RBC. There was no significant effect of the starting pH_e on the magnitude of acidification caused by noradrenaline over the pH_e ranges 7.56–7.83 and 7.31–7.99 for yellowfin and skipjack tuna blood, respectively. However, the magnitude of acidification (i.e., ΔpH_e) was significantly different between the two species: 0.157 ± 0.011 ($n = 10$) and 0.266 ± 0.018 ($n = 8$) for yellowfin and skipjack tuna blood, respectively.

The relationship between pH_e and pH_i in yellowfin tuna blood before and after exposure to catecholamines is shown in Fig. 3. The distribution of H^+ across the RBC membrane in the noradrenaline-treated cells is different from the normal Donnan distribution in the control group. This is due to the rate of Na^+/H^+ exchange being significantly greater than that for Cl^-/HCO_3^- exchange following the addition of catecholamines.

The effects of noradrenaline and ion transport inhibitors on yellowfin tuna RBC are shown in Fig. 4. As previously noted, noradrenaline acidified pH_e. However, pH_i did not consistently increase and was not significantly different ($P > 0.05$) from control pH_i. Incubation of RBC with amiloride, a blocker of Na^+/H^+ exchange, did not completely inhibit decreases in pH_e, although the magnitude of the decrease was significantly reduced ($P < 0.05$). The most accentuated changes in pH_e and pH_i occurred in the RBC treated with DIDS plus noradrenaline. DIDS inhibits anion exchange, and the accentuated changes in pH_e and pH_i provide evidence that the usually rapid cycling of HCO_3^- and Cl^- across the RBC membrane was impaired, thus preventing the re-cycling of H^+ equivalents back across the cell membrane (Nikinmaa 1992).

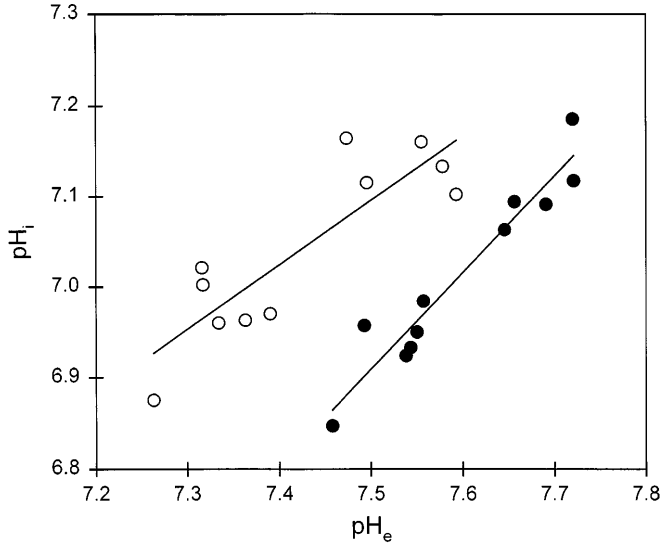

Fig. 3 The relationship between pH_e and red blood cell intracellular pH (pH_i) in yellowfin tuna blood before (*filled circle*) and after (*open circle*) incubation with 10^{-5} M noradrenaline ($n = 10$)

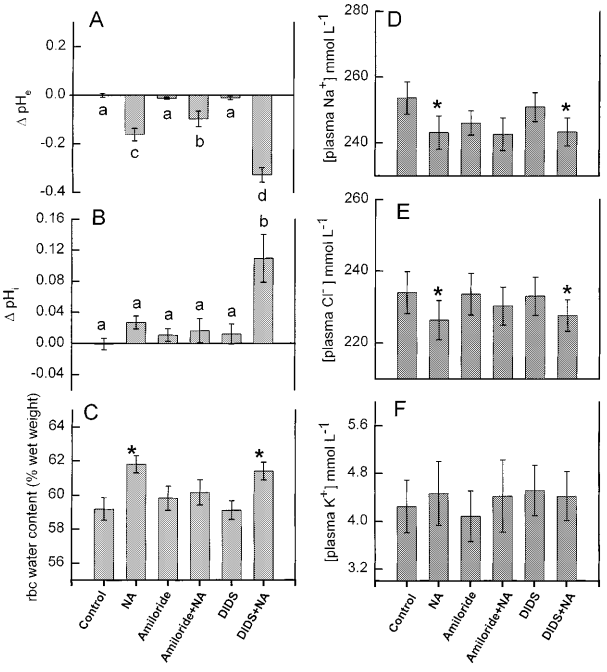

Fig. 4A–F Effect of ion transport inhibitors on the response of yellowfin tuna red blood cells (*rbc*) to noradrenaline. The treatments are: addition of saline (control), noradrenaline, amiloride, amiloride plus noradrenaline, 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (*DIDS*), and DIDS plus noradrenaline. Where significant differences are indicated by *lower case letters*, analysis is by ANOVA. Where significant differences are indicated by an asterisk (*), comparisons are by Students *t*-test. Values that are not significantly different ($P > 0.05$) share common letters. Values are mean ± SEM. In panels **A** and **B**, $n = 6$; in panels **C–F**, $n = 4$

Significant RBC swelling, as shown by a net increase in the RBC cell water content (Fig. 4), occurred in both the noradrenaline and the DIDS plus noradrenaline treatments. RBC swelling is a result of the rapid Na^+ influx and simultaneous H^+ efflux causing a left shift in the $HCO_3^- + H^+ \Longleftrightarrow CO_2 + H_2O$ equilibrium. This stimulates HCO_3^- efflux and Cl^- influx by the membrane bound ("Band 3") anion exchanger. Plasma [Na^+] and [Cl^-] decreased significantly following the addition of noradrenaline in both the control and DIDS treatments, which is consistent with the mechanism described above for cell swelling (i.e., [Na^+] and [Cl^-] both increase within the cell). There was no significant change in plasma [K^+] in any of the treatments.

Effects of catecholamines on blood O_2 binding

Representative blood O_2 dissociation curves are presented in Fig. 5. The control pH_e values shown are the minimum and maximum for the skipjack and yellowfin tuna blood used in our study. For both species, noradrenaline did not affect O_2 binding in any more than a minor way, even at the lowest pH_e when blood O_2 binding curves are plainly shifted right. Clearly, for tuna blood in vitro, catecholamines appear unable to counteract the effects of metabolic acidosis.

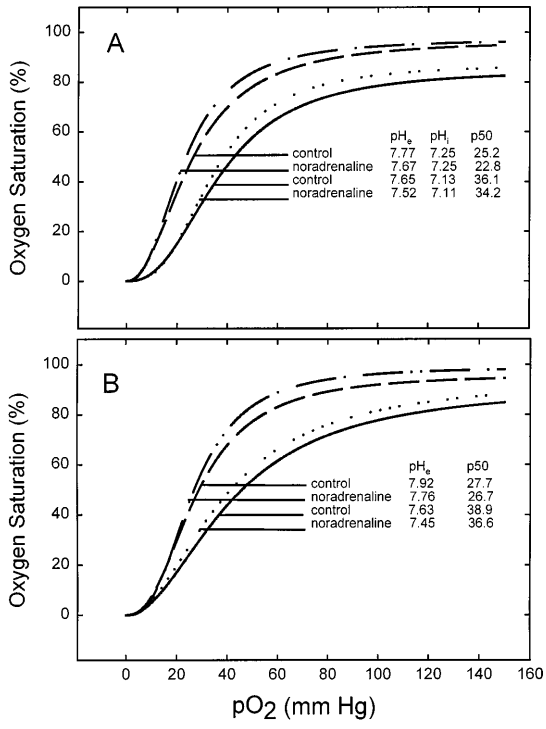

Fig. 5 Effects of noradrenaline (10^{-5} M) on blood O_2 dissociation curves (constructed at 1% CO_2) for **A** yellowfin and **B** skipjack tuna. These curves show the range in pH_e of the blood samples used in our study. Plotted data are the results of the logistics curve fit to the measured partial pressure of O_2 (pO_2) and blood O_2 content data

The effects of noradrenaline in skipjack and yellowfin tuna blood, over the range of initial pH_e, are summarized in Table 1. The P_{50} decreased significantly following the addition of noradrenaline in blood from both species, although the magnitude of the change is relatively small. Moreover, both the P_{50} and the change in P_{50} depend on the calculation method. The P_{50} and the change in P_{50} following the addition of noradrenaline are both lower when P_{50} is calculated with the Root effect taken into account. The pH_i in yellowfin tuna RBC was unchanged by noradrenaline. Although pH_i was not measured in skipjack RBC, the similarity of O_2 dissociation curves and the relatively small change in P_{50} following exposure to catecholamines suggest pH_i also remained unchanged. Total O_2 saturation increased slightly in the blood from both species. The addition of noradrenaline resulted in a small increase in the Hill number of yellowfin tuna blood, but had no influence on the Hill number of skipjack tuna blood.

We were also able to calculate the (fixed acid) Bohr effect coefficient and the Root effect by starting with a range of initial pH_e (Table 1). There was no significant difference in the Bohr effect coefficient per se between skipjack and yellowfin tuna blood; nor did the P_{50} calculation method have any influence on the calculated Bohr effect coefficient. Noradrenaline had no significant influence on the magnitude of the Bohr effect coefficient or the Root effect in blood from either tuna species.

The increases in pH_i and pH_e as blood goes from oxygenated to deoxygenated reflect the magnitude of the Haldane effect (and also intracellular and extracellular buffer values). Large changes in pH_i and pH_e with deoxygenation (Fig. 6) imply a significant Haldane effect occurs in both yellowfin and skipjack tuna blood. The major change in pH_e and pH_i happens between approxi-

Table 1 The blood pH at the pO_2 required to reach 50% O_2 saturation (pH_{50}), red blood cell intracellular pH (pH_i) and O_2 binding variables of tuna blood equilibrated with 99% air and 1% CO_2 prior to (control) and following a 30 min incubation with 10^{-5} M noradrenaline. Values are the mean ± SEM. Significant differences before and after exposure to noradrenaline were determined using paired Students *t*-test (P_{50} pO_2 required to reach 50% O_2 saturation, *MCHC* mean cell hemoglobin concentration, *Hb* hemoglobin)

	Skipjack tuna (n = 8)		Yellowfin tuna (n = 10)	
	Control	Noradrenaline	Control	Noradrenaline
[a]P_{50} (mm Hg)	31.7 ± 1.5	30.4 ± 1.4	30.4 ± 1.5	29.2 ± 1.6*
[b]P_{50} (mm Hg)	34.9 ± 2.0	32.6 ± 1.8 *	34.2 ± 2.1	31.4 ± 2.0*
pH_{50}	7.74 ± 0.04	7.58 ± 0.04 *	7.71 ± 0.02	7.56 ± 0.03*
pH_i			7.17 ± 0.03	7.18 ± 0.03
Total O_2 saturation (%)	91.2 ± 1.4	94.0 ± 1.3 *	87.5 ± 1.6	90.4 ± 1.6*
Root effect (Δ%sat · ΔpH^{-1})	31.1 ± 9.5	20.2 ± 8.1	63.3 ± 18.7	50.4 ± 6.1
[c]Bohr effect coefficient	−0.47 ± 0.16	−0.42 ± 0.08	−0.60 ± 0.26	−0.52 ± 0.20
[d]Bohr effect coefficient	−0.58 ± 0.15	−0.49 ± 0.10	−0.88 ± 0.33	−0.72 ± 0.18
Hill number	1.96 ± 0.15	2.05 ± 0.15	1.80 ± 0.07	1.90 ± 0.01*
Hematocrit (%)	34.9 ± 2.0		37.8 ± 1.7	
Hb (g · dl^{-1})	13 ± 0.8		14 ± 0.6	
[e]MCHC (g · dl^{-1})	37 ± 0.2		36 ± 0.2	
[HCO_3^-] (mmol)	12.6 ± 1.8		10.3 ± 0.7	

* Significant difference before and after incubation with noradrenaline ($P < 0.05$)
[a] P_{50} with the Root effect taken into account
[b] P_{50} without the Root effect taken into account
[c] Bohr effect coefficient with Root effect taken into account
[d] Bohr effect coefficient without Root effect taken into account
[e] Mean cell Hb concentration

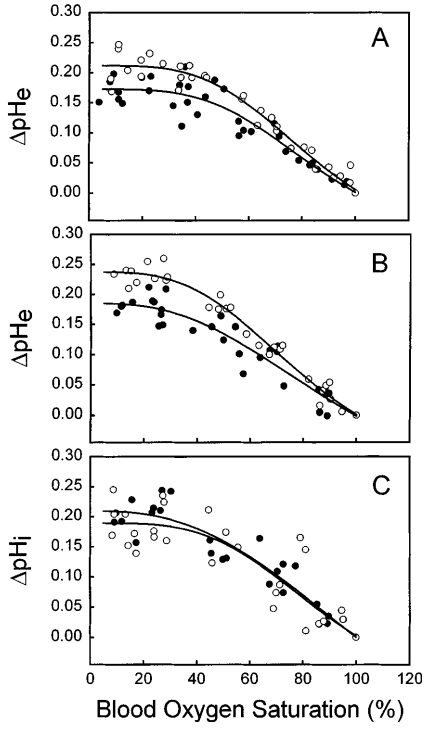

Fig. 6 The effect of blood O_2 saturation and noradrenaline (10^{-5} M) on the change in pH_e (ΔpH_e) accompanying deoxygenation in **A** skipjack blood and **B** yellowfin blood; and **C** the effect of blood O_2 saturation and noradrenaline on the change in pH_i (ΔpH_i) accompanying deoxygenation in yellowfin red blood cells. *Open circles* show data recorded prior to exposure to noradrenaline, *filled circles* show data recorded after 30 min exposure to noradrenaline

mately 40–100% saturation. The change in pH_e with deoxygenation state closely followed changes in pH_i in yellowfin tuna RBC. This may be due to the high hematocrit of tuna blood exaggerating the effect of the transfer of acid-base equivalents across the RBC membrane from a relatively small plasma volume. Noradrenaline (10^{-5} M) significantly enhanced the increase in pH_e accompanying deoxygenation of the blood from both species, although it had no effect on the change in pH_i accompanying deoxygenation in yellowfin RBC (Fig. 6).

Discussion

Tunas are stressed by initial capture and transport to shore-side holding tanks but do recover blood parameters to normal levels somewhere within 5–20 days (Bourke et al. 1981). Tunas are also likely stressed by anesthesia, surgery, and restraint. However, our methods are currently the best available for obtaining tuna blood with normal hematocrit, [Hb], and lactate levels; and with a reasonable range of acid-base status for in vitro experiments. Keen et al. (1995) found plasma levels of adrenaline (5.5×10^{-8} M and 1.7×10^{-7} M for yellowfin and skipjack tuna, respectively) and noradrenaline (3.8×10^{-8} M and 2.5×10^{-7} M for yellowfin and skipjack tuna, respectively) to be relatively high in blood samples taken from tunas handled as we did and restrained under essentially identical conditions. These catecholamine levels are, however, only a fraction (1/10^{th} to 1/30^{th}) of those observed in purposely stressed yellowfin and skipjack tunas, or in yellowfin tuna sampled by cardiac puncture at sea immediately after landing (Watson 1990). Sampling blood at sea from freshly landed tuna clearly is not advantageous and there are no data on blood catecholamine levels in catheterized tunas swimming in tanks or swim tunnels.

Moreover, the majority of catechalomines released due to anesthesia, catheterization, restraint, or blood withdrawal would be degraded by exposure of the blood to bright light (Gilmour et al. 1994). We also ensured complete removal of endogenous catecholamines, and minimized any residual effects of endogenous catecholamines on RBC, by storing the blood overnight at 4 °C (Bourne and Cossins 1982; Roig et al. 1997). Leaving blood overnight permits RBC to regain a steady state with respect to ion and water content (Gallardo Romero et al. 1996; Kaloyianni and Rasidaki 1996). We chose not to use the alternative approach of washing and re-suspending the cells in artificial medium, as this procedure has been shown to influence the affinity and number of catecholamine receptors in trout RBC (Reid et al. 1991), and because the composition of the medium can influence the magnitude of the response of RBC to catecholamines (Dalessio et al. 1991).

The release of stress hormones (i.e., catecholamines and cortisol) and the respiratory conditions present at the time of release (e.g., hypoxia, hypercapnia) both influence the sensitivity and magnitude of the response of salmonid RBC to catecholamines (Reid and Perry 1991; Reid et al. 1993; Perry et al. 1996). The elevation of plasma cortisol levels, hypoxia, and repeated physical stress may up-regulate catecholamine receptors on the RBC membrane (Reid and Perry 1991; Perry et al. 1996). Conversely, prolonged elevation of plasma catecholamines may down-regulate the receptors (Gilmour et al. 1994). During preliminary experiments, we obtained blood from yellowfin tuna which had difficulty surviving the trauma of capture and transport to our holding tanks at the Kewalo Research Facility. These fish were observed swimming close to the side of the tank and appeared physically exhausted. In this situation the animals are killed for ethical reasons. RBC from these animals had a significantly reduced response to catecholamines (unpublished observations). Attempts to measure cortisol in tuna have so far been unsuccessful (R.W. Brill and K.L. Cousins, unpublished observations), but plasma levels of this hormone are most likely elevated in recently captured tunas, as they are in other fishes (Davis and Parker 1986; Pankhurst and Sharples 1992; Pankhurst and Dedual 1994). Thus, it appears stress can affect the responsiveness of tuna RBC to catecholamines. A lack of data prevents us from addressing this question in any detail, however, and the issue clearly needs further study.

Given these caveats, we conclude that a Na^+/H^+ antiporter is present on the tuna RBC membrane and that it is similar to that described for rainbow trout RBC (Baroin et al. 1984; Nikinmaa and Huestis 1984; Cossins and Richardson 1985). The following observations support our conclusion. First, a dose-dependent extracellular acidification occurs following the addition of adrenaline or noradrenaline (Fig. 2). Second, acidification of pH_e and alkalization of pH_i is reduced in the presence of amiloride, whereas there is a pronounced increase in pH_i in the presence of DIDS (Fig. 4). Third, the ratio of pH_i/pH_e changes significantly following the addition of noradrenaline (Fig. 3) indicating that the normal Donnan distribution of protons across the cell membrane becomes uncoupled and that significant changes occur in the active transport of H^+, HCO_3^-, or Cl^- (Nikinmaa 1990).

Changes in plasma ions (Fig. 4) indicate that the action of both amiloride and DIDS may have been incomplete or that other ion transporters or exchanges were operating at the same time. Nevertheless, these results also generally support the presence of a β-adrenergic receptor activated Na^+/H^+ antiporter on tuna RBC, which is comparable to that described for trout RBC. Results similar to ours were obtained for the Atlantic cod (Gadus morhua) RBC, although adrenaline was more effective than noradrenaline in activating the Na^+/H^+ exchanger (Berenbrink and Bridges 1994).

The addition of noradrenaline to tuna blood immediately lowers pH_e, which then slowly rises to a constant level of acidification over 5–10 min (Fig. 1). This is similar to the changes seen in trout blood in vitro (Motais et al. 1989). The rapid decrease in pH_e is due to the accumulation of H^+, because the dehydration of HCO_3^- is not catalyzed (by carbonic anhydrase) in the plasma. A temporary disequilibrium, lasting for approximately a few tens of minutes, thus prevails. We measured reductions in pH_e of 0.16 and of 0.27 for yellowfin and skipjack tuna blood, respectively, and an increase of 0.03 pH_i for yellowfin tuna RBC (Fig. 4). Activation of the Na^+/H^+ antiporter of trout RBC in their own plasma causes a reduction in pH_e of 0.5–0.7 and increase of pH_i of 0.1 (Motais et al. 1989). Cod blood shows a decrease of 0.1 in pH_e and an increase of 0.03 in pH_i (Berenbrink and Bridges 1994). These data appear to imply that the quantity of H^+ extruded from tuna RBC is less than the quantity extruded from trout RBC following exposure to catecholamines, but greater than the quantity extruded by cod RBC.

However, when attempting to discern the presumptive activity of the Na^+/H^+ exchanger based on changes in pH_e, temperature and plasma buffering capacity need to be taken into account. Experiments on trout and cod RBC were conducted at 12–15 °C, whereas our experiments were conducted at 25 °C. Higher temperature will increase the activity of the Na^+/H^+ exchanger, although the rate of HCO_3^- dehydration will also be increased. Therefore, the effect of temperature on the latter will tend to counteract the effect of temperature on the former. More relevant are the buffering capacities of the tuna and trout plasma. Although the absolute difference is small, yellowfin and skipjack tuna plasma do have approximately twice the buffering capacity (5.7 slykes and 4.4 slykes, respectively; Brill et al. 1992; R.W. Brill and K.L. Cousins, unpublished observations) of trout plasma (2.6 slykes; Wood et al. 1982). Therefore, the quantity of H^+ extruded following exposure to catecholamines may indeed be similar in tuna and trout RBC.

Yellowfin and skipjack tuna blood have approximately twice the non-bicarbonate buffering capacity (20.9 slykes and 22.4 slykes, respectively; Brill et al. 1992; R.W. Brill and K.L. Cousins, unpublished observations) of trout blood (10.3 slykes; Wood and Jackson 1980) due mainly to higher [Hb] (yellowfin tuna 12 $g \cdot dl^{-1}$; skipjack tuna 13–14 $g \cdot dl^{-1}$; rainbow trout 5.4 $g \cdot dl^{-1}$; Brill and Bushnell 1991a; Wells and Weber 1991; R.W. Brill and K.L. Cousins, unpublished observations; Table 1). Mean cell [Hb] is also higher in yellowfin tuna (35–37 $g \cdot dl^{-1}$, Brill and Jones 1994; Table 1) and skipjack tuna RBC (36–37 $g \cdot dl^{-1}$; Table 1) than trout RBC (28 $g \cdot dl^{-1}$, Wells and Weber 1991) and may partly explain the modest change seen in pH_i in yellowfin tuna blood. However, our pH_i data are also in agreement with lower rates of H^+ efflux from tuna RBC following exposure to catecholamines.

The sensitivities of teleost RBC to adrenaline and noradrenaline are listed in Table 2. The range of in vivo plasma concentrations of adrenaline and noradrenaline in captive swimming tunas or in the wild is not known. The maximum adrenaline and noradrenaline concentrations in blood sampled from restrained fish are 5.5×10^{-8} M and 3.8×10^{-8} M (respectively) in yellowfin tuna and 1.7×10^{-7} M and 2.5×10^{-7} M (respectively) in skipjack tuna (Keen et al. 1995). Clearly, the sensitivity of tuna RBC to noradrenaline is within the range reported for trout and cod, and the Na^+/H^+ exchanger on tuna RBC would be stimulated maximally by reasonable catecholamine levels observed in vivo. These data suggest a significant, albeit not unusual, role for catecholamines in RBC pH_i regulation in tunas.

Salama and Nikinmaa (1989) suggest the variation in the RBC sensitivity to catecholamines is related to activity levels, with more active species having the greatest response. Although tuna RBC show a distinct physiological response to catecholamines, the response is not clearly greater than that seen in trout RBC. However, of the two tuna species, the response was greater in skipjack tuna RBC. There are a number of physiological differences between skipjack and yellowfin tuna (reviewed in Bushnell and Jones 1994). For instance, the standard metabolic rate of skipjack tuna is nearly twice that of similar sized yellowfin tuna (Brill 1987; Bushnell and Brill 1992). Anatomical differences, such as the lack of a swimbladder and shorter pectoral fins, cause skipjack tuna to have a higher minimum swimming speed than yellowfin tuna (Magnuson 1978). Our findings imply that the sensitivity of the RBC response to cate-

Table 2 Comparison of the sensitivity of red blood cells of various fishes to catecholamines. All values are for normoxic conditions (EC_{50} drug concentrations producing a response 50% of the maximal response)

Species	Catecholamine	EC_{50} values calculated from dose response curves	Reference
Yellowfin tuna	Adrenaline	9.6×10^{-7} M[a]	
	Noradrenaline	8.9×10^{-8} M[a]	
Skipjack tuna	Adrenaline	7.0×10^{-7} M[a]	
	Noradrenaline	4.8×10^{-8} M[a]	
Rainbow trout	Adrenaline	7.6×10^{-7} M[a]	Tetens et al. 1988
	Noradrenaline	1.3×10^{-8} M[a]	
Rainbow trout	Adrenaline	$3.0 \pm 1.1 \times 10^{-7}$ M[a]	Cossins and Kilbey 1989
	Noradrenaline	$2.3 \pm 3.0 \times 10^{-8}$ M[a]	
Rainbow trout	Noradrenaline (control)	2.9×10^{-7} M[b]	Perry et al. 1996
	Noradrenaline (chased)	8.1×10^{-8} M[b]	
Atlantic cod	Adrenaline	4.7×10^{-8} M[a]	Berenbrink and Bridges 1994
	Noradrenaline	1.4×10^{-7} M[a]	

[a] EC_{50} values from dose response curves determined by changes in true plasma pH
[b] EC_{50} values from dose response curves determined by changes in cell volume

cholamines increases with activity within a closely related group of teleosts, but that important evolutionary, environmental, or physiological constraints vary the response between more distantly related species. An alternative hypothesis is that salmonid RBC have an especially pronounced response to catecholamines (Salama and Nikinmaa 1989). Support for either hypothesis must await further comparative work on a larger number of teleost species.

Effects of catecholamines on blood O_2 binding

Exhaustive exercise in skipjack and yellowfin tunas can decrease pH_e by 0.4 units (Perry et al. 1985; Brill et al. 1992). Based on the data for the fixed acid Bohr effect and Root effect given in Table 1 and Fig. 5, following exhaustive exercise P_{50} would increase from approximately 26 mm Hg (at pH_e 7.8) to approximately 40 mm Hg (at pH_e 7.4) in skipjack and yellowfin tuna in the absence of catecholamines. With catecholamines, the change in P_{50} would be reduced only slightly; P_{50} would still increase to approximately 37–38 mm Hg. Similarly, at pH_e 7.4 without catecholamines, blood $[O_2]$ at a pO_2 of 150 mm Hg is 75–88% of its theoretical maximum based on [Hb]. At pH_e 7.4 but with exposure to catecholamines, blood O_2 carrying capacity would be only slightly higher – approximately 80–92% of its theoretical maximum based on [Hb]. Thus, while catecholamines can effect tuna blood O_2 transport, the reduction in blood O_2 binding caused by metabolic acidosis can be only partially offset by catecholamines. Our hypothesis, that the importance of catecholamines in maintaining blood oxygen transport is enhanced in tunas relative to other teleosts, is not supported by our data.

The importance of catecholamines for regulation of pH_i and blood O_2 transport in fish is highly variable. For example, Thomas and Perry (1992) clearly demonstrated that catecholamines are important in rainbow trout, whereas Tufts et al. (1991) found that catecholamines had only a modest effect on the pH_i/pH_e gradient across the RBC of "wild" Atlantic salmon (Salmo salar). Tufts et al. (1991) concluded that increased [Hb] and arterial pO_2 were more important than the effects of catecholamines on RBC during recovery from exhaustive exercise in this species. Our findings imply the same is true for maintaining or increasing O_2 delivery during recovery from exhaustive exercise in skipjack and yellowfin tunas. Tunas have maximum metabolic rates far greater than those of other fishes, and even approach those of mammals (Brill and Bushnell 1991b; Bushnell and Jones 1994). Obviously, high metabolic rates can only be supported by high rates of O_2 delivery to the tissues. As discussed by Brill and Jones (1994), elevated blood O_2 carrying capacity is achieved in yellowfin tuna by a high mean cell [Hb] concentration, which increases blood O_2 carrying capacity without a concomitant increase in blood viscosity. Also, splenic contraction is common in active fish species during exhaustive exercise (Yamamoto et al. 1980). While routine hematocrits in tunas range from approximately 30–40% (Bushnell and Jones 1994), hematocrits above 50% and [Hb] above 20 $g \cdot dl^{-1}$ are seen in tunas under stress (Klawe et al. 1963; Laurs et al. 1978; Wells et al. 1986; R.W. Brill, unpublished observations).

In summary, our data indicate tuna RBC do not have an enhanced response to catecholamines, and that catecholamines do not necessarily increase or even maintain blood O_2 affinity or O_2 transport following exhaustive exercise. Rather, as first suggested by Brill and Bushnell (1991b), the ability to increase hematocrit, cardiac output, arterial-venous blood [O2] difference, and effective gill surface area appear to be the essential characteristics that tunas have evolved for achieving the high rates of blood O_2 transport necessary for rapid recovery from exhaustive exercise. Moreover, although there are no data on cardio-respiratory function in tunas during recovery from exhaustive exercise, the changes in cardio-respiratory function seen in tunas during acute hypoxia (Bushnell and Brill 1992) and moderate levels of

aerobic exercise (Korsmeyer et al. 1996b, 1997a,b) support our supposition.

Effect of catechalomines on CO_2 excretion

Catecholamines inhibit CO_2 excretion in trout, although the exact mechanism of inhibition is controversial (Perry et al. 1991; Wood and Perry 1991; Wood and Simmons 1994). One theory is that the internal RBC pool of H^+ is competed for by both the catecholamine-stimulated Na^+/H^+ exchanger and the intracellular HCO_3^- dehydration reaction (Randall and Brauner 1991). In both skipjack and yellowfin tuna blood, the decrease in pH_e accompanying incubation with noradrenaline decreases with increasing O_2 saturation (Fig. 6). These data imply that fewer H^+ ions are available for the Na^+/H^+ exchanger as Hb O_2 saturation increases, and that catecholamines may also indeed limit CO_2 excretion in tunas.

In contrast, in tuna RBC noradrenaline had no measurable effect on pH_i at any level of blood O_2 saturation (Fig. 6). There are two possible explanations for the lack of an effect of noradrenaline on pH_i. First, catecholamine-stimulated Na^+/H^+ exchange is reduced in yellowfin tuna RBC. Second, the intrinsic buffer capacity of yellowfin tuna Hb is larger at any given oxygenation state than is the Hb from other teleosts. Recent data (F.B. Jensen, personal communication) have shown, however, that the buffering capacities of purified skipjack and yellowfin tuna Hb (in both the oxygenated and deoxygenated conformations) are relatively low, as they are in teleost Hb in general (Jensen 1991). Furthermore, the fixed acid Haldane effect (i.e., the change in pH_i with oxygenation due to H^+ release from Hb during oxygenation) in tuna Hb is large ($\Delta pH_i = 0.20$, Fig. 6C), but equivalent to that seen in rainbow trout ($\Delta pH_i = 0.21$–0.24; Jensen 1986; Brauner et al. 1996) and tench ($\Delta pH_i = 0.35$; Jensen 1986). The decrease in pH_i accompanying oxygenation depends on both the quantity of protons released from the Hb and the buffer value of the Hb (Jensen 1986), and both appear roughly equivalent in tuna, trout and tench. The stable pH_i in tuna following incubation with noradrenaline, therefore, implies that a lack of H^+ within the RBC would not limit CO_2 excretion in the presence of catecholamines in vivo. We therefore speculate that the inhibition of CO_2 excretion by catecholamines may be restricted to salmonids. In species like yellowfin tuna, which show a comparatively reduced effect of catecholamines on pH_i but an equivalent fixed acid Haldane effect, inhibition of CO_2 excretion by catecholamines may not be a significant problem.

Importance of the Haldane and Bohr effects in yellowfin tuna for O_2 and CO_2 transport

In contrast to O_2, which is bound to the Hb, the majority of CO_2 is hydrated to HCO_3^- and is transported in the plasma in fishes (Perry et al. 1982). This reaction occurs slowly at an uncatalyzed rate in the plasma, but at a rapid catalyzed rate within the RBC. The resulting HCO_3^- is exchanged for Cl^- by the membrane bound (''Band 3'') anion exchanger (Motais et al. 1989; Jensen 1991). The Bohr and Root effects are dependent on pH_i. Therefore, the relative buffering capacity of the Hb is important for O_2 and CO_2 transport (i.e. O_2 and CO_2 transport are inexorably linked) (Brauner and Randall 1996; Nikinmaa 1997). In teleost Hb, the buffer capacity at a given degree of oxygenation is relatively low compared to mammalian and elasmobranch Hb (Jensen 1991; Perry et al. 1996). The low buffer capacity of teleost Hb, and consequently the potential problem of excess H^+ ions within the RBC, is compensated for by having a large Haldane effect. In other words, in teleost blood the ability of Hb to buffer H^+ is greatly altered by a change in oxygenation. As O_2 is unloaded at the capillaries, the buffering capacity of Hb increases, which minimizes changes in pH_i. The large change in pH_i and pH_e in both yellowfin and skipjack tuna blood, going from the deoxygenated to oxygenated state at constant CO_2, confirms the presence of a large Haldane effect (Fig. 6). The largest changes in the pH_i and pH_e occur between approximately 40–100% O_2 saturation, which are similar to changes recorded in tench and rainbow trout blood (Jensen 1986; Brauner et al. 1996).

Due to the linkage of the Haldane and Bohr effects, it is not possible to exploit an optimal Bohr shift ($\Delta P_{50}/\Delta pH$) and (simultaneously) a maximal Haldane effect. In other words, a large Haldane effect (i.e., H^+ uptake by the Hb upon deoxygenation) will limit the change in pH_i when CO_2 is added to the blood in the capillaries. This, in turn, reduces O_2 offloading from the Hb due to the Bohr shift. Jensen (1991) suggests, therefore, that the relative magnitudes of the Haldane and Bohr effects indicate whether a fish species favors pH and CO_2 homeostasis or O_2 affinity change (i.e., O_2 unloading at the tissues). The relatively high values for the CO_2 Bohr effect coefficients in tuna blood (−0.83 to −1.17; Bushnell and Jones 1994) reported previously suggest an emphasis on CO_2 transport compared with rainbow trout (−0.49; Tetens and Christensen 1987). On this basis it would seem that trout blood is better adapted to O_2 transport, whereas tuna blood is more adapted to CO_2 transport. However, the fixed acid Bohr effect coefficients for yellowfin and skipjack tuna blood found when the Root effect is taken into account (−0.60 and −0.47, respectively) are closer to that of trout. The relative importance of pH and CO_2 homeostasis versus O_2 affinity change in tuna blood therefore remains an open question.

As extensively discussed by Brauner (1995) and Brauner and Randall (1996), the physiological significance of the Haldane and Bohr effects for CO_2 transport becomes apparent when previous cardiovascular and respiratory studies on tuna are re-examined. Data from Jones et al. (1986), Bushnell and Brill (1992), and Korsmeyer et al. (1996a, b) indicate that during normoxia

and low levels of activity, tuna blood shows a decrease in pH_e during transit through the gills. A similar change in pH_e occurs in rainbow trout at low exercise levels. The decrease in pH_e upon oxygenation is due to the greater release of H^+ from Hb than there are HCO_3^- ions available for dehydration. This situation changes with hypoxia or exercise and the concomitant increase in arterial – venous blood $[O_2]$ difference. At higher levels of arterial – venous blood $[O_2]$ difference, pH_e increases as blood transits the gills in trout (solid line, Fig. 7) (Brauner 1995; Brauner et al. 1996). To date, the highest sustainable swimming speeds reported for captive and instrumented tuna are 1.8–2.9 body lengths $\cdot s^{-1}$ in kawakawa (*Euthynnus affinis*) (Jones et al. 1986). The maximum sustainable speed recorded in a swim tunnel is approximately 2 body lengths s^{-1} (Korsmeyer et al. 1996a, b, 1997a). The arterial – venous blood $[O_2]$ difference and pH_e change occurring in yellowfin tuna blood during its transit through the gills have also been plotted in Fig. 7. It appears that changes in pH_e in tunas are also a function of O_2 demand. In other words, in tunas the relationship of arterial to venous blood $[O_2]$ difference, and the arterial to venous pH_e difference, is similar to that observed in rainbow trout even though tunas have much higher rates of blood O_2 delivery.

At low swimming speeds, or in paralyzed tunas, venous blood returns to the gills at approximately 50% O_2 saturation (Bushnell and Brill 1992; Korsmeyer et al. 1997b), a situation similar to that seen in trout (Eddy 1976; Kiceniuk and Jones 1977). This indicates that both tunas and trout fully exploit the Haldane effect for CO_2 excretion in the gills under these circumstances, because of the large release of Bohr protons when the Hb is oxygenated (Brauner and Randall 1997). In tunas, as in other teleosts, venous blood O_2 saturation falls with increases in activity and hypoxia (Kiceniuk and Jones 1977; Bushnell and Brill 1992; Korsmeyer et al. 1997b). Therefore, although there are far fewer data for tunas than for other teleosts, the non-linear release of Bohr protons with oxygenation seen in tuna blood (Fig. 6) most likely serves the same physiological functions during hypoxia and exercise in tunas as Brauner and Randall (1997) eloquently describe it doing so for other fishes.

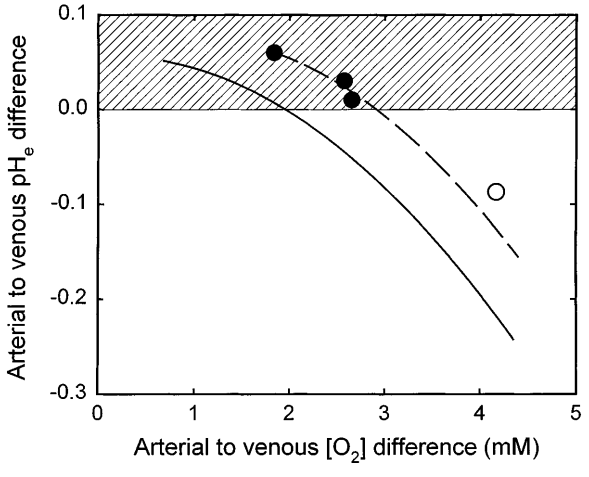

Fig. 7 The effects of blood oxygenation on pH_e in vivo. The *solid line* shows the change in pH_e occurring as the blood traverses the gills (i.e., arterial to venous pH_e difference) in rainbow trout (Brauner 1995), the *broken line* shows the equivalent data for yellowfin tuna. The abscissa is the change in blood O_2 content ($[O_2]$) as blood traverses the gills (i.e., arterial to venous $[O_2]$ difference) and thus reflects O_2 demand (i.e., metabolic rate). The *solid circles* are data from yellowfin tuna swimming 1–1.83 body lengths $\cdot s^{-1}$ (Korsmeyer et al. 1996a, b), the *open circles* are data from kawakawa swimming at 1.8–2.9 body lengths $\cdot s^{-1}$ (Jones et al. 1986)

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