A comparison of adrenergic stress responses in three tropical teleosts exposed to acute hypoxia

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Perry, S. F., S. G. Reid, K. M. Gilmour, C. L. Boijink, J. M. Lopes, W. K. Milsom, and F. T. Rantin. A comparison of adrenergic stress responses in three tropical teleosts exposed to acute hypoxia. Am J Physiol Regul Integr Comp Physiol 287: R188–R197, 2004.—Experiments were performed to assess the afferent and efferent limbs of the adrenergic stress response in selected tropical species during acute hypoxia. The ventilatory responses to hypoxia in each species were similar, consisting generally of increases in both frequency and amplitude. These responses were not synchronized with or influenced by plasma catecholamine (Cat) secretion. The ventilatory responses to hypoxia in each species occurred as blood O2 concentration was reduced to approximately 50–60% of the normoxic value. Intravascular injections of nicotine elicited pronounced increases in plasma Cat levels. Thus the acute humoral adrenergic stress response elicited by exposure to environmental hypoxia (see Table 1 in Ref. 39). From these studies it is clear that all species that have been examined exhibit an elevation of plasma catecholamine levels during hypoxia. However, the degree of hypoxia required to initiate the responses is highly variable. For example, hypoxia-tolerant species such as eel (Anguilla rostrata; Ref. 25) or carp (Cyprinus carpio; Ref. 45) require exposure to a severe level of hypoxia to initiate the humoral adrenergic stress response and at such times display only modest elevations of circulating catecholamines. In contrast, hypoxia-intolerant species such as rainbow trout (Oncorhynchus mykiss; Ref. 3) or cod (Gadus morhua; Ref. 9) tend to secrete greater quantities of catecholamines, and the onset of the response appears to occur at higher water PO2 (Pw02) levels. Although relatively few species have been examined, these variable responses may be related to differences in Hb-O2 binding affinities. Thus, while catecholamine release might be initiated at widely different levels of Pw02 or arterial PO2 (Pa02), this threshold appears to occur after a similar reduction of arterial blood O2 concentration corresponding to ~50% Hb O2 saturation. Consequently, it has been suggested that the threshold for catecholamine release during hypoxia occurs at a PaO2 that is roughly equivalent to the P50 of Hb (i.e., PaO2 at 50% Hb-O2 saturation) (25, 26, 34, 39).

Many tropical fish species have evolved in environments that exhibit spatial and temporal fluctuations in ambient O2 levels. Exposure to intermittent hypoxia, in particular, has led to numerous morphological, physiological, and behavioral adaptations that serve to optimize O2 transfer and blood O2 transport (10, 41). One such adaptation, the presence of high-affinity Hbs, facilitates blood O2 transport at the low PaO2 levels.
values that are characteristic of fish inhabiting hypoxic waters. Consequently, if a 50% reduction in Hb–O2 saturation were indeed a reliable predictor of the onset of the acute humoral adrenergic stress response, catecholamine release in such tropical fish exposed to acute hypoxia would be expected to occur at low PaO2 values roughly equivalent to their oxyhemoglobin P50s. Surprisingly, however, plasma catecholamine levels have not been measured in any tropical species exposed to hypoxia (or any other stressor). Thus, although numerous studies have addressed the physiological consequences of elevated circulating catecholamines in tropical fish (13, 14, 43) and while it has been suggested that an elevation of plasma catecholamine levels may be a key factor promoting their adaptive responses to hypoxia (44), it is not yet known whether these fish actually release catecholamines into the circulation at such times.

Therefore, in the present study, plasma catecholamine levels and blood respiratory status were measured during acute aquatic hypoxia in three tropical teleost species belonging to the same taxonomic order (Characiformes), the obligate water breathers Hoplias malabaricus (traira) and Pirapactus mesopotamicus (pacu) and the facultative air breather Hoplerythrinus unitaeniatus (jeju). We hypothesized that in each species, catecholamine release would occur at a PaO2 level equivalent to the P50 of its Hb.

MATERIALS AND METHODS

Experimental Animals

Adult specimens of Hoplerythrinus unitaeniatus (jeju), weighing 225 ± 13 g (mean ± SE, n = 55), were collected from the Paraná River basin near Bataguassu, State of Mato Grosso do Sul. Adult specimens of Hoplias malabaricus (traira), weighing 253 ± 24 g (n = 32), were collected from the Monjolinho reservoir, campus of the Federal University of São Carlos, and in the Rio Mogi Guacu basin, near the Jatia Ecological Station, Luiz Antônio, SP, Brazil. Specimens of juvenile (2–3 yr old) P. mesopotamicus (pacu) weighing 705 ± 44 g (n = 24) were obtained from the Center of Research on Tropical Fish (CEPTA-IBAMA)-Pirassununga, SP, Brazil. After transportation to the laboratory, all fish were maintained outdoors under natural photoperiod in 500- to 1,000-liter tanks supplied with aerated non-chlorinated water at 25 ± 1°C (acclimation temperature). Jeju and traira were fed weekly with live food (smaller live fish of various species), whereas pacu were fed daily with commercial pellets; in all cases food was withheld for 2–3 days before trials.

Surgical Procedures

Fish were anesthetized in a solution of benzocaine (ethyl-p-amino-benzoate; 0.1 g/l). After cessation of breathing movements, the fish were transferred to an operating table, and the gills were irrigated for the duration of the surgery with a more dilute anesthetic solution (0.5 g/l) gassed with O2. To allow periodic blood sampling and injection of nicotine (see below), a cannula (Clay-Adams PE-50 polyethylene tubing) was inserted into the caudal artery according to standard surgical procedures (1). Briefly, a lateral incision (~2 cm in length) was made at the level of the caudal peduncle ~3 mm below the lateral line, the epaxial muscle was cut to expose the vertebral, and the artery was cannulated in the retrograde direction by percutaneous puncture. The incision was sutured using a running stitch, and the cannula was then secured to the body wall with silk ligatures. Using a Dremel tool, a hole was drilled through the snout between the nostrils and a flap of muscle was raised. Using a Dremel tool, a hole was drilled through the snout between the nostrils and a flap of muscle was raised. The incision was sutured using a running stitch, and the cannula was then secured to the body wall with silk ligatures. Using a Dremel tool, a hole was drilled through the snout between the nostrils and a flap of muscle was raised.

For some experiments (series 3), impedance electrodes were sutured to each operculum to measure the breath-by-breath displacement of the operculum to allow continuous monitoring of ventilation rate (fV) and ventilation amplitude (VAMP).

After surgery, the animals were manually ventilated with aerated water, and as soon as they showed signs of revival, they were placed into individual cylindrical tubes housed within larger experimental tanks. Mesh covered the ends of the cylindrical tube. This facilitated rapid equilibration of the water within the tube with the water in the holding tank. A large slit on top of the tubes permitted the impedance leads/cannulas to exit the tank. The tank was covered to maintain a dark and undisturbed environment for the fish. Animals were allowed to recover for a minimum of 24 h before experimentation in normoxic water (PaO2 ≥ 130 mmHg) at their acclimation temperature (25°C). Each experimental series utilized different groups of animals.

Experimental Protocols

Series 1: blood gasses and stress hormones during hypoxia. After a recovery period of ~24 h, the fish (n = 14 for pacu; n = 22 for traira; n = 17 for jeju), within their boxes, were submerged in a large volume of water (500 liters) contained in an opaque covered tank. After 2–3 h, the fish were subjected to progressive hypoxia and serial blood sampling. Fish were first exposed to hypoxia and then subjected to three of six levels of increasing hypoxia (60, 50, 40, 30, 20, or 10 mmHg). This protocol, in which any particular fish was subjected only to a subset of the hypoxic PaO2 target levels, helped to minimize blood loss. Before any experiment, the flow of fresh water was stopped and the remaining water was recirculated vigorously. Hypoxia was achieved by gassing the lower part of the covered tank with controlled amounts of N2. The PaO2 was continuously monitored by the electrode of a FAC-204A O2 analyzer. To ensure constant levels of hypoxia, the O2 analyzer was connected to a controller that opened or closed a solenoid valve to vary the flow of N2 appropriately. Owing to the large reservoir of water (500 liters) relative to fish mass and the short duration of the experiments (<2 h), it is unlikely that there were any appreciable changes in CO2 or ammonia levels in the recirculating water.

On reaching the target PaO2, a blood sample (0.7 ml) was withdrawn and analyzed immediately for hematocrit (Hct), Hb values, total O2 concentration (CaO2), and PaO2. After centrifugation (10,000 × g for 5 min) of the remaining blood, the plasma was removed, frozen in liquid N2, and then stored at −80°C. The red blood cells (RBCs) were resuspended in saline and reinserted into the caudal artery cannula.

To assess the capacity of each species to secrete catecholamines, fish (n = 10 for pacu; n = 11 for traira; n = 8 for jeju) were injected intra-arterially with 600 nmol/kg of nicotine (1 ml/kg), a potent catecholamine secretagogue, 30–60 min after their return to normoxia. Three blood samples (0.5 ml) were taken from each fish, an initial sample followed by samples at 2 and 5 min postinjection.

Series 2: air breathing in jeju during hypoxia. Air-breathing frequency (breaths/hour) and air-breathing duration (total time spent air breathing) were recorded in a separate group of jeju (n = 10) using a custom-designed experimental setup (21a). The upper part of the experimental chamber consisted of an “inverted funnel,” the neck of which housed an electric bulb positioned in front of a photoelectric cell. To breathe air, fish were forced to pass through the neck, interrupting the light circuit between the bulb and the photocell. This interruption was detected by a decoder/amplifier in which a square wave was generated and recorded by a data-acquisition system (DI 154 Datagu Instrument).

After at least a 24-h recovery period, the fish (n = 10 for each species) were transferred, within its cylindrical tube, to the experimental chamber (~10 liters). Water flow through the chamber was at a rate of 1,600 ml/min, and again the tank was covered to maintain a dark and undisturbed environment for the fish. The opercular impedance leads were connected to an impedance converter to measure fV.
To produce hypoxia, N2 was bubbled into the water where it entered the experimental chamber. The PwO₂ was allowed to fall rapidly at first (3–4 mmHg/min) until the PO₂ had fallen to ~60 mmHg, where it was maintained for 15 min. The PO₂ was then decreased further a 10 mmHg over a 5-min period and maintained at this new level for a further 10 min. This procedure was repeated in 10-mmHg steps down to a PO₂ of 10 mmHg. After a full 10 min at the final level, the PO₂ was returned to starting levels, the fish was euthanized, and the body weight was recorded.

Analytical Procedures

Blood samples (40 µl) were analyzed for O₂ content (CaO₂) using an OXYCON Blood O₂ Content Analyzer (Cameron Instruments). Hb concentration was determined in duplicate on 20-µl samples using a commercial spectrophotometric Hb assay kit (Sigma). Hct was determined in duplicate by centrifuging microcapillary tubes at 5,000 g for 10 min. PaO₂ was measured by injecting ~100 µl of blood into the sample compartment of an O₂ electrode (Cameron Instruments) housed within a thermostatted (25°C) cuvette (Radiometer).

Plasma samples and standards for catecholamine analysis were subjected to alumina extraction in Brazil and then shipped on dry ice to Ottawa for analysis by HPLC with electrochemical detection (47).

3,4-Dihydroxybenzylamine was used as an internal reference standard in all analyses. Detection limits for Epi and NE were 0.1 nmol/l. Plasma cortisol levels were measured on duplicate 20-µl samples using a commercial RIA kit (ICN).

Construction of O₂ Equilibrium Curves

O₂ specifically bound to Hb (O₂/Hb; mol O₂/mol Hb) was calculated after subtraction of physically dissolved O₂ in the plasma; O₂ capacitance coefficients for human plasma were obtained from Boutillier et al. (4). [O₂]/[Hb] was plotted against PaO₂, and a sigmoidal curve (3-parameter Hill equation) was fitted to the data using a commercial graphics software package (SigmaPlot 8.0); 100% Hb-O₂ saturation values were obtained from these curves, allowing O₂ equilibrium curves to be expressed as a function of percentage Hb-O₂ saturation.

Statistical Analysis

The data are reported as means ± SE. Time series data were compared using one-way repeated-measures ANOVA. If significant differences (P ≤ 0.05) were found, Tukey’s (series 3) or Bonferroni (series 1 and 2) multiple comparison tests were applied. In some cases (e.g., see Fig. 6), the statistical difference between two means was assessed using a nonpaired Student’s t-test. For all statistical analyses, a commercial software package was used (SigmaStat 2.03; SPSS).

RESULTS

Series 1: Blood Gasses and Stress Hormones During Hypoxia

Blood respiratory, hematological, and hormone variables in fish before hypoxia exposure are depicted in Table 1. Aside from low PaO₂ and CaO₂ in jeju denied access to air, there were no significant differences among the three species. Importantly, for the purposes of this study, plasma catecholamine levels (the sum of Epi and NE) were low and averaged about 2.0–3.5 nmol/l (Table 1). In pacu and traira, the predominant catecholamine was NE, whereas in jeju, there were roughly equivalent quantities of Epi and NE. Only in one species (traira) did serial blood sampling cause a significant reduction in Hct (from 19.1 ± 1.1 to 14.6 ± 0.8% after the 4th blood sample; data not shown).

The construction of in vivo O₂ equilibrium curves (Fig. 1) revealed that while each species possessed relatively high-affinity Hbs, there was also significant variation among the species with Pso values ranging between 11.3 (pacu) and 7.7 mmHg (jeju).

Plasma catecholamine levels remained constant in pacu during hypoxia even at PwO₂ levels as low as 10 mmHg (Fig. 2). In traira and jeju without access to air, plasma catecholamine concentrations were elevated at PwO₂ below 20 and 40 mmHg, respectively. The predominant circulating catecholamine in traira exposed to hypoxia was NE, whereas the more abundant catecholamine in hypoxic jeju was Epi (data not shown). Figure 3 depicts the relationships between plasma catecholamine levels and either PaO₂ (Fig. 3, A–C) or Hb-O₂ saturation (Fig. 3, D–F). In traira and jeju, catecholamine release into the circulation occurred at abrupt thresholds corresponding to PaO₂ values of approximately 8.5–12.5 mmHg. Given the Pso values of 8.6 and 7.7 in traira and jeju, respectively, one would expect catecholamine release to be initiated as Hb-O₂ saturation fell below 50–60%. Indeed, the data in Fig. 3, E and F, demonstrate that catecholamine release in traira and jeju occurred as blood O₂ concentration was reduced to approximately 50–60% of the normoxic value. Although distinct thresholds for catecholamine release were demonstrated for traira and jeju (Fig. 3), some fish did not experience an increase in plasma catecholamine levels despite displaying Pso well below the threshold levels.

To assess the potential capacity for catecholamine secretion in each species, fish were injected with nicotine, a known activator of secretion in vertebrates. Traira and jeju experienced significant increases in plasma catecholamine levels after nicotine injection, whereas there was no nicotine-evoked increase in pacu (Fig. 4).

In jeju allowed access to air, plasma catecholamine levels remained constant even at PwO₂ as low as 10 mmHg (Fig. 5A). In these fish, Pao₂ was markedly elevated compared with fish

Table 1. Blood or plasma levels before the initiation of hypoxia in 3 tropical species

<table>
<thead>
<tr>
<th>Species</th>
<th>PaO₂, mmHg</th>
<th>[O₂], nmol/l</th>
<th>[Hb], nmol/l</th>
<th>O₂/Hb, mol/mol</th>
<th>Hematocrit, %</th>
<th>NE, nmol/l</th>
<th>Epi, nmol/l</th>
<th>Total Cat, nmol/l</th>
<th>Cortisol, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacu</td>
<td>100±2.5</td>
<td>77.9±3.6</td>
<td>4.2±0.2</td>
<td>1.18±0.06</td>
<td>22.7±1.2</td>
<td>3.1±0.9</td>
<td>0.21±0.05</td>
<td>3.4±0.9</td>
<td>1.9±0.7</td>
</tr>
<tr>
<td>Traira</td>
<td>77.9±3.6</td>
<td>10.2±3.3</td>
<td>3.03±0.3</td>
<td>0.81±0.05</td>
<td>19.1±1.1</td>
<td>1.5±0.8</td>
<td>0.4±0.2</td>
<td>1.9±0.7</td>
<td>3.3±1.8</td>
</tr>
<tr>
<td>Jeju</td>
<td>77.9±3.6</td>
<td>10.2±3.3</td>
<td>3.03±0.3</td>
<td>0.81±0.05</td>
<td>19.1±1.1</td>
<td>1.5±0.8</td>
<td>0.4±0.2</td>
<td>3.3±1.8</td>
<td>3.3±1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; (n), no. of fish. 3 of the tropical species are the obligate water breathers Hoplias malabaricus (traira) and Piaractus mesopotamicus (pacu) and the facultative air breather, Hoplopython unirnaiuenticus (jeju). PaO₂, arterial Po₂; Hb, hemoglobin; NE, norepinephrine; Epi, epinephrine; Cat, catecholamines.
that were denied access to air (Fig. 6). Thus, compared with the jeju that were held exclusively in water, relatively few PaO₂ values in those with access to air (6 vs. 31) fell below the threshold required to elicit catecholamine secretion (compare Fig. 5, B and C).

Series 2: Air Breathing in Jeju During Hypoxia

In a separate series of experiments performed on uncanullated fish, air-breathing frequency (Fig. 7A) and proportion of time spent breathing air (Fig. 7B) were monitored during normoxia and progressive hypoxia. Air breathing was not detectable under normoxic conditions but became increasingly prevalent as PwO₂ was lowered below 40 mmHg. At the most severe level of hypoxia (10 mmHg), fish were averaging 36 breaths/hour and spending ~10 min of each hour breathing air.

Series 3: Aquatic Ventilatory Responses of Fish to Hypoxia

Each species displayed a hyperventilation response during hypoxia with variable increases in fV and VAMP (Fig. 8). In pacu (Fig. 8, A and B) and traira (Fig. 8, C and D), the hyperventilation was statistically significant only at the most severe levels of hypoxia (<30 mmHg). In jeju (Fig. 8, E and F), hyperventilatory responses were observed at PwO₂ of 50 mmHg and below. However, in jeju, the hyperventilation was transient with ventilation returning to normoxic values at the most severe levels of hypoxia (Fig. 8, E and F). By comparing the ventilatory responses to hypoxia with the intervals of catecholamine release (depicted as horizontal lines in Fig. 8), it is clear that hyperventilation occurred before, or in the total absence of, plasma catecholamine elevation. Moreover, the

Fig. 2. Plasma catecholamine (Cat; the sum of epinephrine plus norepinephrine) levels in pacu (A), traira (B), and jeju (C) without access to air. Each fish was exposed to normoxia (N) and then 3 of 6 levels of hypoxia spanning 10–60 mmHg. *Statistically significant differences (P < 0.05) from the normoxia values.

Fig. 1. In vivo O₂ equilibrium curves for *Piaractus mesopotamicus* (pacu; A), *Hoplias malabaricus* (traira; B), and *Hoplerythrinus unitaeniatus* (jeju; C) obtained by measuring arterial P O₂ (PaO₂) and arterial O₂ concentration (CaO₂) during exposure of fish to progressive hypoxia. For each species, the PaO₂ at which the Hb was 50% saturated with O₂ (P₅₀) was determined from Hill plots (not shown).
abrupt secretion of catecholamines into the circulation was not associated with any obvious additional hyperventilation, and indeed in jeju, there appeared to be a decrease in breathing during the period of plasma catecholamine elevation (Fig. 8, E and F).

**DISCUSSION**

Numerous studies have examined aspects of the acute humoral adrenergic stress response in fish exposed to hypoxia, yet data on plasma catecholamine levels exist for relatively few species. Of these, the majority of data have been obtained from rainbow trout, a species that is unlikely to encounter hypoxia in the natural environment. Although other examined species may inhabit hypoxic water [e.g., eel (25) and hagfish (2)], they are unlikely to experience rapid changes in PwO₂, and thus may not exploit an acute adrenergic stress response in nature. Numerous tropical fish, however, routinely inhabit waters that experience massive spatial and/or temporal changes in PwO₂ (41, 44). In addition to seasonal changes, temporal drift on a daily basis can lead to rapid fluctuations of several hundred millimeters Hg over a period of only hours (42). Such rapid changes in the O₂ status of the water are known to promote a host of physiological adjustments that, in part, are thought to be initiated by increased levels of circulating catecholamines (44). The present study, however, provides the first data on plasma NE and Epi levels in any tropical fish and demonstrates that catecholamine release into the circulation is not required to trigger short-term adjustments to hypoxia. Moreover, by conducting these experiments on species possessing high-affinity Hb, and by comparing responses in a facultative air breather (jeju) with or without access to air, we provide compelling evidence to support a largely untested model (25) that contends that blood O₂ status is the key variable controlling catecholamine secretion in hypoxic fish.

**Catecholamine Release During Hypoxia is Controlled by Blood O₂ Status**

Based on available evidence, catecholamine secretion in fish exposed to acute hypoxia is delayed until the PaO₂ is lowered sufficiently to reduce Hb-O₂ saturation to approximately 50–60% (25, 26). Consequently, the PaO₂ threshold for catecholamine release is believed to be related to the O₂-binding affinity of Hb (22). In the present study, the three tropical species that were examined displayed relatively high-affinity Hbs with P5₀ values ranging between 7.7 and 11.3 mmHg. RBC pH, arguably the key determinant of Hb-O₂ binding affinity, and whole blood pH (a reliable indicator of RBC pH) were not measured in the present study. Thus, in the absence of such measurements, we cannot attribute the differences in the P5₀ values among the species to differences in pH. Previous studies (reviewed in Ref. 22) have demonstrated that changes in blood pH in teleost fish do not contribute directly to catecholamine secretion. Thus, despite the absence of pH measurements in this study, we are confident that the intraspecific differences in catecholamine secretion cannot be explained by varying patterns of blood pH change during hypoxia. In the two species that exhibited catecholamine secretion during hypoxia (traira and jeju), the PaO₂ thresholds were roughly equivalent to the P5₀ values. Indeed, if these new data.
are plotted together with previous results from studies assessing catecholamine secretion in trout or eel (25–27, 40), a clear relationship emerges (Fig. 9) in which the Pa O2 catecholamine release threshold is positively correlated with P50 (R2 = 0.87; P < 0.05).

Although a reduction to a threshold level of Hb-O2 saturation or a closely related variable such as CaO2 is required to elicit a humoral adrenergic stress response, the precise pathways evoking catecholamine secretion remain unknown. Recently, cyanide-sensitive branchial O2 chemoreceptors linked to systemic catecholamine secretion were identified in rainbow trout (35). These receptors are oriented both externally and internally and thus are potentially able to initiate catecholamine secretion on adequate reduction of either external (water) or internal (blood) O2 status. However, in rainbow trout, the internally oriented receptors appear to be more important because catecholamine release during hypoxia was unaffected by selective removal of the external receptors (35). A comparison of the responses of hypoxic jeju with or without access to air provides further evidence that the internal O2 status is the principal variable controlling catecholamine release. In that particular experiment, even when exposed to the most severe levels of hypoxia (10 mmHg) and displaying regular air breathing, the fish were spending >80% of the time submerged. Thus external O2 chemoreceptors surely were being stimulated regardless of whether fish were breathing air. The likeliest explanation for the absence of catecholamine release in the fish breathing air is that PaO2 was protected and thus did not reach the threshold for mobilization. Although jeju denied access to air were prevented from performing the natural behavior of air breathing, we do not believe that catecholamine release in these fish was a result of generalized stress. Indeed, plasma cortisol levels, even at the most severe level of hypoxia (10 mmHg), were not increased in hypoxic jeju without air access.

**Fig. 5.** A: plasma total catecholamine (cat) levels (the sum of epinephrine plus norepinephrine) in jeju exposed to acute hypoxia spanning 10–60 mmHg. Fish were either denied access to air (filled bars) or allowed to breathe air (open bars). *Statistical differences (P < 0.05) from the normoxia values; † statistical differences (P < 0.05) between the fish with or without air access at any level of hypoxia. PwO2, water PO2. B and C: relationships between PaO2 and plasma total catecholamine levels in jeju denied access to air (B) or allowed free access to air (C). The horizontal double-headed arrow depicts the range of PaO2 over which catecholamine release occurred in the fish denied access to air.

**Fig. 6.** Relationships between PwO2 and PaO2 in jeju during normoxia (>100 mmHg) and subsequent acute hypoxia spanning 10–60 mmHg. Fish were either denied access to air (filled circles or bars) or allowed to breathe air (open circles or bars). A: data from individual fish. B: means after separation of the data into 4 discrete groups (normoxia and 3 levels of hypoxia). *Statistical differences (P < 0.05) from the normoxia values; † statistical differences (P > 0.05) between the fish with or without air access at any level of hypoxia.
(35.3 ± 6.4 ng/ml during normoxia vs. 47.0 ± 11.2 ng/ml during severe hypoxia; P > 0.05). Moreover, were catecholamine release occurring as a generalized stress response (even in the absence of a cortisol response), one would not expect to see a distinct PaO2 threshold for catecholamine secretion. Thus the linkage of catecholamine release to variations in intracellular nucleoside triphosphate levels (for review, see Ref. 30). Therefore, the linkage of catecholamine release to variations in intracellular nucleoside triphosphate levels is consistent with a role for circulating catecholamines in regulating blood O2 levels.

Although we were able to identify thresholds for catecholamine release in traira and jeju, in several cases, catecholamine levels in the circulation remained low even after PaO2 or Hb-O2 had passed well beyond these thresholds. Similar intraspecific variation was reported previously for rainbow trout (23). Although we cannot explain the mechanistic basis for this intraspecific variation in the onset of catecholamine secretion during hypoxia, it is consistent with the idea that elevated circulating catecholamines are not required to initiate many of the rapidly occurring responses to hypoxia.

Arguably, the most significant effect of catecholamine release during hypoxia is to enhance blood O2 transport by increasing carrying capacity (46, 48) and by enhancing Hb-O2 binding affinity via alkalization of the RBC (6, 17) and reductions in intracellular nucleoside triphosphate levels (for review, see Ref. 30). Thus the linkage of catecholamine release to internal O2 status and in particular a reduction of Hb-O2 to 50–60% saturation prevents premature elevation of plasma catecholamine levels and ensures that catecholamines are secreted at a time when the blood O2 capacitance is highest (at the P50). In other words, the humoral adrenergic stress response is primed to begin as the PaO2 reaches a zone of high capacitance (steep portion of the O2 equilibrium curve) where small changes in PaO2 can have dramatic effects on arterial O2 content. It is far more difficult to envisage a tight linkage between plasma catecholamine levels and blood O2 status if the catecholamine secretion response was instead initiated by external O2 chemoreceptors. Indeed, the external O2 chemoreceptors are stimulated at much higher values of PO2 and largely serve to regulate cardiorespiratory function.

**Absence of Catecholamine Secretion During Hypoxia in Pacu**

Although attenuated catecholamine secretion responses to severe physical stress (e.g., capture, chasing, surgery) have been reported in Antarctic teleosts (7, 8), to our knowledge, pacu is the only fish species so far examined that does not exhibit an elevation of circulating catecholamines during acute hypoxia. It is unlikely that the absence of catecholamine release in this species reflected an inadequate degree of hypoxia because PaO2 and Hb-O2 saturation fell to levels that normally would be expected to initiate secretion in other species. Although it is feasible that catecholamine release might have occurred in pacu had PwO2 been lowered even further (below 10 mmHg), the physiological significance of such a delayed response (given the marked fall in Hb-O2 saturation) would seem to be inconsistent with a role for circulating catecholamines in regulating blood O2 levels.

In the majority of vertebrate species studied to date, including fish, it appears that the nicotinic receptor is the predominant cholinergic receptor present on chromaffin cells (see Ref. 34) and is also largely responsible for catecholamine secretion during sympathetic stimulation. Thus injection of nicotine, in vivo, can be used as an effective tool to assess the potential capacity of the chromaffin tissue (if present) to secrete catecholamines. Similar to the situation in rainbow trout (12), injection of nicotine into the caudal artery of traira or jeju caused marked increases in circulating catecholamine levels. The fact that pacu was unresponsive to this high dose of nicotine (600 nmol/kg) suggests an inoperative or absent humoral adrenergic stress response in this species. Alternatively, the chromaffin tissue, if present, is insensitive to nicotine, leading to the possibility that catecholamine secretion in this species is activated by a fundamentally different pathway than in other vertebrates. Clearly, further research is required to differentiate among these possibilities.

**Asynchrony Between Ventilatory Adjustments and Catecholamine Secretion During Hypoxia**

The results of the present study do not support the suggestion that elevated circulating catecholamines play a critical role in the initiation of short-term cardiorespiratory adjustments of tropical fish exposed to hypoxia. In these experiments, only the ventilatory response to hypoxia was monitored, but it is unlikely that the conclusion of a lack of involvement of circulating catecholamines would not apply to cardiovascular changes. In short, the ventilatory (this study) and cardiovascular (38) responses are initiated by mild levels of hypoxia, whereas catecholamine release commences only when severe levels of hypoxia are achieved (e.g., <40 mmHg; this study). Conse-
quently, circulating catecholamines, if playing a role, can only do so during severe hypoxia, long after compensatory responses have already begun. Whether the release of catecholamines during severe hypoxia aids the hyperventilatory response is difficult to ascertain. The possibility that circulating catecholamines may have a role in stimulating ventilation in fishes has been the subject of considerable debate with evidence being presented both for (31) and against (24) their role. In the present study, there was no increase in ventilation during the period of catecholamine release, and indeed the largest increase in breathing frequency during the period of severe hypoxia (PwO$_2$ < 40 mmHg) occurred in pacu, the one species that did not release catecholamines. On the other hand, in traira and jeju, breathing frequency was actually reduced during the period of catecholamine release. It has been argued (24) that hypoventilatory responses associated with circulating catecholamines may reflect sudden increases in blood O$_2$ content owing to activation of RBC Na$^+$/H$^+$ exchangers (18). In turn,
the rapid improvement in blood $O_2$ status could serve to relieve in part the drive on internal $O_2$ chemoreceptors. Presently, it is not known whether the RBCs of traira or jeju (species that release catecholamines during hypoxia) exhibit adrenergic responses. In a survey of the two largest orders of Amazonian teleosts, Val et al. (43) reported that RBC adrenergic responses were present in representatives of the Characiformes. Thus traira and jeju, both members of the Characiformes (10), conceivably could possess adrenergically responsive RBCs.

**Perspectives**

The results presented in this paper are consistent with an arguably controversial view (20) that the involvement of circulating catecholamines in promoting compensatory responses during hypoxia is less significant than previously believed. Clearly, the rapidly occurring cardiorespiratory adjustments to hypoxia can in no way be related to circulating catecholamines. By occurring after a severe reduction in blood $O_2$ content, catecholamine release is likely to have an impact on blood $O_2$ transport and metabolism only under the direst of circumstances. Indeed, although few data exist, it is possible that the release of catecholamines into the circulation during hypoxia is somehow linked to the critical $P_{O_2}$ (the $P_WO_2$, at which there is an abrupt transition from regulation of to a lowering of metabolic rate). For example, the critical $P_{O_2}$S in traira and jeju are 20 mmHg (32) and 40 mmHg (13a), respectively. It is unclear whether the rise in circulating catecholamines precedes the critical $P_{O_2}$ and thus may be responsible (in part) for the sudden metabolic transition, or alternatively occurs only after the critical $P_{O_2}$ has been reached. In the latter case, the increase in circulating catecholamines may be aimed at countering the decrease in metabolic rate through an effect on blood $O_2$ transport.

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