The interactive effects of hypoxemia, hyperoxia, and temperature on the gill morphology of goldfish (*Carassius auratus*)

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Submitted 13 August 2010; accepted in final form 10 March 2011

**Tzaneva V, Bailey S, Perry SF.** The interactive effects of hypoxemia, hyperoxia, and temperature on the gill morphology of goldfish (*Carassius auratus*). *Am J Physiol Regul Integr Comp Physiol* 300: R1344–R1351, 2011. First published March 16, 2011; doi:10.1152/ajpregu.00530.2010.—Acclimation of crucian carp to temperatures below 15°C causes covering of the gill lamellae by a mass of cells termed the interlamellar cell mass (ILCM). Here we explore the cues underlying gill remodeling (removal or growth of an ILCM) and specifically test the hypotheses that 1) depletion of internal O2 stores in the absence of any change in external O2 status can trigger the removal of the ILCM in goldfish acclimated to 7°C; 2) exposing fish acclimated to 25°C to an abundance of O2 (hyperoxia) can reverse the gill remodeling (i.e., cause the covering of lamellae by an expansion of the ILCM), and 3) neuromodulatory cells (NECs) are involved in signaling the shedding of the ILCM. Hypoxemia induced by phenylhydrazine (anemia) or 5% CO caused a decrease in the ILCM from 80% to 23% and 35%, respectively. Hyperoxia at 25°C caused an increase to 67% of total ILCM and a smaller decrease in the size of the ILCM when fish were transferred from 7 to 25°C. Daily sodium cyanide injections were used to stimulate NECs; this treatment led to a significant decrease in the ILCM. Thus, the three major conclusions of this study are 1) that gill remodeling can occur during periods of internal hypoxemia, 2) that O2 supply and demand may be a significant driving force shaping gill remodeling in goldfish, and 3) the NECs may play a role in triggering the shedding of the ILCM during hypoxia.

A VARIETY OF EXTERNAL and/or internal cues are known to alter the morphology of the gill epithelium in fish inhabiting freshwater. For example, exposing rainbow trout (*Oncorhynchus mykiss*) to soft (low Ca\(^{2+}\) concentration) water, (26) or ion-poor water (25) induces the proliferation of mitochondrion-rich cells or ionocytes, also referred to as chloride cells (22), which may be tested in a thickening of the blood-to-water diffusion distance (11; for a review, see Ref. 23). Similar changes in gill morphology can be elicited by treatment of fish with hormones such as cortisol (18) or growth hormone (20) without any associated change in the external environment. Another example of gill remodeling occurs in at least two closely related cyprinid species, crucian carp (*Carassius carassius*) and goldfish (*C. auratus*), which exhibit pronounced structural modification of the gill in response to changes in ambient temperature or O2 levels (22, 36, 37, 38). For example, at temperatures > 15°C the goldfish gill contains protruding lamellae but upon acclimation to colder water (e.g., 7–8°C) the lamellae become largely embedded in a mass of cells that has been termed the interlamellar cell mass (ILCM) (36). It is believed that the covering of the lamellae in fish acclimated to colder water serves to reduce the energetic costs of osmoregulation because passive salt and water fluxes across the gill can be minimized. However, when crucian carp (36) or goldfish (19, 20) that have been acclimated to cold water are exposed to hypoxia, the ILCM is removed, thus exposing the previously embedded lamellae and increasing the functional surface area for gas (and ion exchange). An increase in temperature also acts as a cue for the loss of the ILCM, which may be related to the higher demand for O2 as the metabolic rate increases (8).

The specific cues for gill remodeling in crucian carp or goldfish have not been investigated. However, the fact that increasing temperature and hypoxia both lead to a loss of the ILCM and the uncovering of previously covered lamellae, suggests that increasing O2 demand or a need to maximize O2 uptake may be common prerequisites for gill remodeling. By analogy to the hypoxic hyperventilatory response (24), the removal of the ILCM associated with exposure of cold-acclimated fish to hypoxia could be triggered by the stimulation of external O2 chemoreceptors (4, 16) or by activation of internally oriented receptors responding directly or indirectly to changing O2 levels (for reviews see Refs. 3 and 9). Here we explore the cues underlying gill remodeling and specifically test the hypothesis that depletion of internal O2 stores (hypoxemia) in the absence of any change in external O2 status, can trigger the removal of the ILCM in goldfish acclimated to 7°C. To evoke hypoxemia under continuing conditions of external normoxia, fish were either treated with phenylhydrazine (PHZ) to induce anemia (15) or carbon monoxide (CO) to prevent the binding of O2 to hemoglobin (14). In an attempt to dissociate the effects of temperature, per se, on gill remodeling from the associated changes in O2 demand, fish were exposed to hyperoxic conditions. Specifically, we tested the hypothesis that exposing fish to hypoxia would lead to the formation of an ILCM in warm-acclimated fish and prevent/reduce the uncovering of gill lamellae that normally accompanies the gradual acclimation of goldfish from 7 to 25°C. We also tested the hypothesis that branchial neuroepithelial cells (NECs; O2 chemoreceptors) (16) might play a role in signaling the removal of the ILCM during hypoxia; this was achieved by assessing the effects of sodium cyanide (NaCN) injections (to activate the NECs) on gill morphology.

**MATERIALS AND METHODS**

*Experimental Animals and Surgical Procedures*

Goldfish were purchased from a commercial supplier (Aleong’s International, Mississauga, ON, Canada) and were held in circular tanks supplied with aerated dechloraminated City of Ottawa water at 18°C. One group of goldfish was acclimated to 7°C and another group was acclimated to 25°C by dropping or raising the water temperature...
by ~2°C per day. The fish were held at their respective temperatures on a 12:12-h light-dark photoperiod and were fed commercial pellets for at least 2 wk prior to experimentation. The experiments were carried out at the University of Ottawa Aquatic Care Facilities at the above mentioned temperatures in compliance with guidelines of the Canadian Council of Animal Care and after the approval of the University of Ottawa Animal Care Committee (protocol BL-226).

Large goldfish (average mass = 168 g; n = 8) acclimated to 7°C were fitted with caudal artery cannulae. The cannulation procedure is explained in detail in Tzaneva and Perry (40). Briefly, the fish were anesthetized with 1 g/l benzocaine (Sigma-Aldrich, St. Louis, MO), an ~2-cm incision was made in the caudal peduncle region, and a PE-50 cannula with a 2 cm polyethylene (PE)-10 extension was inserted into the caudal artery. The incision was closed with silk sutures, and the fish were transferred to transparent tanks (2.75 liters) provided with well-aerated flow-through water to recover for 24 h before experiments commenced.

**Experimental Protocol**

CO exposure. Small goldfish acclimated to 7°C (average mass = 21.7 g; n = 24) were placed in two 9-liter transparent tanks receiving aerated flowing water. After 24 h, a commercial mixture of 5% CO in air (Linde Canada, Montreal, QC, Canada) was bubbled into the tanks. Water PO2 remained at normoxic levels throughout the experimental period. Fish were euthanized by an overdose of benzocaine (1 g/l; Sigma-Aldrich) and gill tissue was collected for ILCM analysis at 1, 2, 3, and 7 days after beginning CO exposure. Large cannulated goldfish were kept in transparent tanks (2.75 liters) for 24-h recovery prior to CO exposure. Fish were exposed to CO for 24 h after which blood samples were taken to measure the arterial partial pressure of oxygen (Pao2), the total O2 content of the arterial blood (CatO2), and hematocrit. After removing 0.5–1.0 ml of blood, Pao2 was measured by injecting a 0.2 to 0.3 ml sample of whole blood into the sample chamber of an O2 microelectrode (Microelectrodes, Bedford, NH) attached to a blood gas meter (model BGM 200; Cameron Instruments). The O2 microelectrode was calibrated prior to each use using a two-point calibration method. Zero P02 solution (0.02 g/ml sodium sulfite) and a gas mixture of 18% O2 in N2 gas were used as the low-end calibration point, while air saturated water was used to set the high-point calibration. The gas meter was connected to a data integration system (BioPac), and the data were recorded by AcKnowledge software. CatO2 was measured using a blood O2 content analyzer (Oxycon, Cameron Instruments). The instrument was calibrated by injecting 20 μl of air into the sample chamber. A 50-μl blood sample was injected into the sample chamber containing a solution of 0.3 g saponin and 0.6 g of potassium ferricyanide [K4Fe(CN)6] per 100 ml of distilled water. Total hemoglobin content of the blood was determined using a microplate spectrophotometric assay in which triplicate 20 μl samples of whole blood were added to 5 ml of Drabkin’s solution (Sigma-Aldrich). After 15 min, the absorbance of 200 μl aliquots at 540 nm was measured using a SpectraMax 340PC and SOFTMax PRO software (Molecular Devices).

PHZ exposure. Small goldfish acclimated to 7°C (average mass = 32.1 g; n = 6) were placed in 9-liter transparent tanks supplied with aerated flowing water for 24 h prior to PHZ exposure. After the 24-h acclimation period, the water flow was stopped and the fish were exposed to PHZ (4 mg/l) for 48 h. Air was bubbled into the tanks to keep the water normoxic and the tank was kept in a 7°C water bath to maintain constant temperature during the period of exposure. After 48 h water flow was returned to the tank at a rate of between 1.1 and 2 ml/s, and the fish were allowed to recover for 7 days. Blood samples (< 0.1 ml) were obtained by caudal puncture using a heparinized 0.5-ml syringe and a 23-gauge needle to measure hemoglobin concentration and hematocrit. Control fish (average mass = 28.5 g; n = 6) were kept in 9-liter tanks receiving aerated flowing water for 2 wk after which the gill tissue and blood samples were collected for ILCM analysis and hematocrit and hemoglobin measurements.

Repeated NaCN injections. After inserting a cannula (PE-50, ID = 0.023 in, OD = 0.038 in) through the snout into the buccal cavity, fish were transferred to opaque boxes (1-liter volume) and left to recover for 24 h. Three groups (6 fish/group) of fish (average mass = 28.2 g; n = 18) were injected with 20 mg/kg of either water or NaCN (2 mg/kg) 5 times a day at 1.5-h intervals between 10 AM and 6 PM. NaCN was used to activate branchial O2 chemoreceptors under normoxic conditions. A third group of fish was not injected to determine whether the presence of the cannula, itself, affected the dimensions of the ILCM.

**Hyperoxia exposure.** Six groups of goldfish (average mass = 33 g; n = 36) were used for this experiment. Each group was held in a 9-liter transparent tank supplied with aerated flowing water. Three groups of fish were exposed to hyperoxia (P02 > 500 mmHg). Two of the groups remained at their respective acclimation temperature of 7 or 25°C for the duration of hyperoxia exposure (2 wk), while the temperature of the water providing the third group was increased from 7 to 25°C at a rate of ~2°C per day; fish remained at 25°C for a further 10 days. Control fish were either held at 7 or at 25°C for 2 wk under normoxic conditions or subjected to a temperature change from 7 to 25°C under normoxic conditions. Hyperoxic conditions were achieved by bubbling the water flowing through a water-air equilibration column with O2 using a mass flow controller (Mass Flow Trak, Sierra Instruments,) at a rate of 500–1,000 ml/min until a P02 > 500 mmHg was reached. At the end of the exposure, gill tissue was collected, and the area of the ILCM was measured.

**ILCM analysis.** The first right gill arch from each fish was removed and fixed in 4% paraformaldehyde until it was ready for sectioning. Twenty-four hours before sectioning, the gills were placed in 30% sucrose (wt/vol) solution to dehydrate and cryoprotect the tissue. Prior to sectioning they were placed in OCT Cryomatrix (Thermo Scientific) for at least 1 h before being frozen at −30°C. The tissue sample was then sliced in 12- to 14-μm thick sections using a Leica CM3050 cryostat and stained with hematoxylin (Harris Hematoxylin with Glacial Acetic Acid; Poly Scientific) to visualize the tissue. Light microscopy was used to take photos of six gill filaments randomly per section (36 photos per fish). An Axioskop (Zeiss, Germany) microscope with an Olympus DP70 digital camera were used to obtain the images and the regular cross-sectional area of the ILCM was determined using Image Pro version 6.0 (Media Cybernetics, Bethesda, MD) and Image-J software (http://rsbweb.nih.gov/ij/index.html).

**Statistical Analysis**

Data are represented as means ± 1 SE. The unpaired two-tailed Student’s t-test was used to detect significant differences between goldfish acclimated to 7°C (ILCM present) and 25°C (ILCM absent). The Mann-Whitney U-test was used to detect differences between means when groups did not exhibit equality of variance.

**RESULTS**

Effects of CO and PHZ Exposure on the Size of the ILCM

Exposing fish acclimated to 7°C to 5% CO caused a marked decrease in the mass of the ILCM during the first 48 h of exposure after which there were no further decreases (Fig. 1). In control fish, the ILCM occupied 78.1 ± 5.9% of the total interlamellar area; after 24 and 48 h, the ILCM had decreased by 10.2 ± 3.3% on October 22, 2017 http://ajpregu.physiology.org/ Downloaded from
samples were taken for analysis of CaO2, PaO2, and hematocrit; the data are summarized in Table 1. While there was a pronounced decrease in the total O2 levels of the blood after 24-h CO exposure, PaO2 and hematocrit remained unchanged (Table 1).

A separate group of goldfish was exposed to PHZ for 48 h and was allowed to recover for 7 days. In these fish, the ILCM was reduced to a mere 23.1 ± 6.9% of total interlamellar area (Fig. 2A). Hemoglobin concentration fell significantly from 8.9 ± 1.3 mg/dl in control fish to 1.6 ± 0.2 mg/dl in PHZ-treated fish (Fig. 2B), and hematocrit decreased from 25.0 ± 2.7 to 0.4 ± 0.3% (Fig. 2C). These results confirm that the goldfish exposed to PHZ were indeed anemic.

Figure 3 shows representative micrographs depicting the morphology of the gill in control goldfish (Fig. 3A) and goldfish exposed to either PHZ (Fig. 3B) or CO (Fig. 3, C and D). The images illustrate a noticeable decrease in the size of the ILCM after either CO or PHZ exposure. A single representative image of a goldfish gill is shown after 48 h of CO exposure only (Fig. 3D), because the ILCM did not decrease further after that time.

Table 1. The total O2 content of arterial blood (CaO2), arterial Po2, PaO2, and hematocrit of goldfish acclimated to 7°C before and after 24-h exposure to 5% carbon monoxide (CO)

<table>
<thead>
<tr>
<th>CO Exposure</th>
<th>Before</th>
<th>After 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaO2, mmol/l</td>
<td>1.95 ± 0.39 (7)</td>
<td>0.04 ± 0.05 (6)*</td>
</tr>
<tr>
<td>PaO2, mmHg</td>
<td>125 ± 9.9 (8)</td>
<td>115 ± 5.7 (8)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>18.0 ± 2.2 (7)</td>
<td>18.9 ± 1.7 (8)</td>
</tr>
</tbody>
</table>

Data are shown as means ±1 SE; Numbers per group are in parenthesis. *Significant differences (P < 0.05).

Although food intake was not quantified in this study, feeding appeared to decrease in fish with lowered O2 content in both the CO and PHZ treatments. The overall level of activity of the fish also appeared to decrease after/during CO and PHZ exposures. While the ventilation was not quantified, it did appear that there was a slight hyperventilation in CO-treated fish, at least initially. There was no observable change in ventilation in the PHZ-treated fish.
Effects of Chronic NaCN Injections on the Size of the ILCM

Repeated NaCN injections caused a significant decrease in the ILCM from $78.1 \pm 2.4\%$ to $47.6 \pm 4.7\%$ ($P < 0.001$) (Fig. 4). Uninjected fish and fish injected with water did not show a significant change in their ILCM compared with control goldfish ($P = 0.956$ and 0.978, respectively). There was no significant difference found between cannulated fish that were not injected and those injected with water ($P = 1.0$).

Effects of Hyperoxia Exposure on the Size of the ILCM

The exposure to hyperoxia of fish acclimated to $25^\circ C$ caused a significant increase in the size of the ILCM from $36.0 \pm 2.7$ to $67.7 \pm 2.8\%$ of the total interlamellar area (Fig. 5).
remodeling caused by hyperoxia was so extensive that there was no longer any difference in the average size of the ILCM between the fish at 7 and 25°C. The size of the ILCM in goldfish acclimated to 7°C was not significantly altered by exposure to hyperoxia (Fig. 5). Goldfish experiencing a temperature increase from 7 to 25°C exhibited a decrease in the size of the ILCM from ~80% (assumed to be based on average values from normoxic fish acclimated to 7°C) to 33.4% of the total interlamellar area (Fig. 5). Imposing an identical temperature change under hyperoxic conditions resulted in a final ILCM size of 45.9 ± 5.3% of the interlamellar area, which was significantly greater than in the normoxic fish (i.e., the extent of gill remodeling with increasing temperature was decreased by hyperoxia treatment; Fig. 5). Representative examples of gill morphology of goldfish exposed to different O2 and temperature treatments are presented in Fig. 6.

DISCUSSION

Hypoxemia as a Cue for Gill Remodeling

Previous studies have shown that a temperature increase can act as a cue for the loss of the ILCM in goldfish, possibly because of an increasing O2 demand as metabolic rate rises (38). Environmental hypoxia, the impact of which can be minimized by increasing O2 transfer efficiency, can also induce a decrease in the ILCM in crucian carp (36) or goldfish (19) acclimatized to cold water. These cues for remodeling, although external in origin, will also modify the internal environment of the fish by increasing the demand for O2 (increasing temperature) or lowering blood O2 content (hypoxia). Thus, from such studies it can be difficult to specifically identify the proximate stimulus (or stimuli) underlying the changes in gill morphology during environmental changes in temperature or O2 levels. In this study, we successfully manipulated the internal environment of the fish while keeping the external environment constant. By exposing the goldfish to PHZ or CO, it was possible to elicit profound hypoxemia without any changes in the O2 levels of the external environment (Table 1 and Fig. 2, B and C). Because hypoxemia coincided with a decrease in ILCM with both treatments, it suggests that even though the fish are sensing normoxic levels of O2 in the external environment, the lowering of blood O2 carrying capacity can specifically act as a cue for gill remodeling. The specific mechanisms linking a decrease in arterial blood O2 content to gill remodeling were not specifically investigated in the present study, although it is tempting to draw parallels with current models of control of breathing in teleosts (see below). However, one factor that can be excluded as a contributing variable is PaO2, because it was unchanged during CO exposure (Table 1). Although PaO2 was not measured in the anemic fish in the present study, previous studies on rainbow trout (5, 10) have demonstrated that anemia is without effect on PaO2. While there is some evidence that

Fig. 6. Representative photomicrographs illustrating the gill morphology of goldfish (C. auratus) exposed to hyperoxia acclimated to 25°C (B) or 7°C (D) or undergoing a temperature increase from 7 to 25°C (F) and in normoxic control fish acclimated to 25°C (A) or 7°C (C) or undergoing a temperature increase from 7 to 25°C (E). Scale bars represent 50 μm.
hypercapnia may elicit gill remodeling in goldfish (J. Bradshaw and S. F. Perry, unpublished observations), it is unlikely that changes in arterial blood CO2 levels were a contributing factor to remodeling in the present study because PaCO2, while likely increasing during anemia (10), would presumably be unaffected by CO exposure (13). Although Holeton (13) reported an absence of any change in blood pH associated with acute (30 min) CO exposure in rainbow trout, it is conceivable that blood pH was reduced by longer-term CO (or PHZ) treatments in goldfish. The potential involvement of blood pH as a trigger for gill remodeling has yet to be investigated.

The potential role of internal versus external O2 receptors in mediating ventilatory responses to ambient hypoxia has received considerable attention (for a review, see Ref. 24). Indeed, similar to the results of the present study, which focused on gill remodeling, previous experiments designed to assess ventilatory responses demonstrated that rainbow trout exposed to CO (12, 14) or rendered anemic (35) exhibited hyperventilation. These findings led to the concept of internal O2 content receptors linked to cardiorespiratory control (31). The notion of some type of internal O2 content receptor was further supported by the results of a series of experiments that correlated arterial O2 content with catecholamine release in fish experiencing acute hypoxia (28, 29, 32). Those experiments clearly demonstrated that catecholamine release was occurring at a critical PO2 threshold, which varied among fish depending on the hemoglobin O2 affinity. Additional evidence to support hypoxemia as a proximate stimulus for catecholamine secretion was the finding that exposing rainbow trout to hyperoxia prevented catecholamine release normally associated with hypercapnic acidosis (27). In agreement with these previous studies, the results of the present investigation suggest that some type of internal receptor responding to blood O2 content is able to initiate gill remodeling. While the theoretical basis for sensing of blood O2 content is not known, it is conceivable that such receptors could be responding to the rate of O2 delivery, which in turn is influenced by blood O2 content.

Evidence that O2 Chemoreceptors Are Involved in Signaling the Shedding of the ILCM

This is the first study to provide evidence that gill O2 chemoreceptor cells may play a role in triggering the loss of the ILCM when goldfish acclimated to cold water (e.g., 7°C in the present study) are exposed to hypoxic water. Repeated NaCN injections into the buccal cavity were used to activate the chemoreceptors (40), while keeping the external environment normoxic. The result, a significant decrease in the size of the ILCM, implicates the branchial O2 chemoreceptors in signaling the shedding of the ILCM. Using an identical dose, Tzaneva and Perry (40) demonstrated a marked hyperventilatory response of goldfish to single buccal injections of NaCN, which, as in numerous previous studies (e.g., 34), was attributed to specific activation of O2 chemoreceptors. An obvious candidate for such a receptor is the gill neuroepithelial cell (NEC) (1, 2, 17), which is known to function as an O2 sensor in zebrafish, Danio rerio (16, 30), and channel catfish, Ictalurus punctatus (4). Given that NECs are also distributed to the filament and lamellae of goldfish gill (36, 40), the simplest explanation for the current results is that NECs of the gill filament and/or lamellae were stimulated repeatedly by the daily injections of NaCN into the buccal cavity. While the injection of NaCN into the buccal cavity likely caused a preferential activation of externally oriented NECs, it may have also caused internally oriented NECs to be stimulated; the preferential activation of internally facing O2 chemoreceptors in goldfish also causes marked hyperventilation (41). Given that the NECs of the filament are well situated to sense changes in both ambient and blood O2 levels, it is conceivable that these presumptive O2 chemoreceptors are involved in gill remodeling during changes in both external and internal O2 levels as would occur in the natural environment when goldfish (or crucian carp) inhabiting cold water encounter hypoxia. While the current evidence implicates internal hypoxemia as a trigger for gill remodeling, there are no comparable data explicitly implicating external O2 receptors.

Previous studies have shown that environmental hypoxia causes the removal of the ILCM in crucian carp through apoptosis (36). There are two possible sources for the signal, which triggers apoptosis in the ILCM: 1) the cells or a subset of cells of the ILCM are themselves O2 sensors, and/or 2) they receive input from the nearby NECs. The results from this study suggest that there are interactions between the NECs and the cells of the ILCM, which cause their removal. Innervation of cells in the ILCM is unlikely to be a possible signaling source, because it has been shown that the ILCM is not directly innervated (40).

Thermally Induced Gill Remodeling is Influenced by External O2 Levels

Changes in ambient water temperature, while impacting on internal and external temperature receptors also cause predictable changes in metabolic rate (8). Thus, the loss of the ILCM in goldfish transferred from cold to warm water (e.g., 7 to 25°C in Ref. 20) could specifically reflect increasing water and/or internal temperature, or alternatively, be related to increasing metabolic rate. To determine whether the increasing O2 demand associated with increasing metabolism might be contributing to gill remodeling (in an attempt to increase O2 uptake), fish acclimated to 25°C were exposed to hyperoxia. Remarkably, the results demonstrated that the gill morphology of 25°C goldfish exposed to hyperoxia closely resembled that of a 7°C normoxic goldfish with only the distal lamellar regions remaining uncovered and in contact with the water. These results, while not excluding a possible role of temperature sensing in the gill remodeling response, certainly indicate that O2 demand may be an important driving force. Thus, while O2 demand still remained high in the fish acclimated to warm water, the abundance of O2 presumably negated the need to shed the ILCM and uncover the lamellae. This idea is consistent with the results of earlier studies, which demonstrated that hyperoxia was able to increase the thermal tolerance of goldfish (42, 43).

Another possible explanation for the increases in the size of the ILCM in fish exposed to hyperoxia is that it serves to protect tissues from oxidative damage produced by reactive oxygen species. It would be interesting to measure arterial PO2 during exposure of goldfish with branchial ILCM coverage to determine whether the ILCM serves as a barrier to the entry of O2 and thus might limit internal oxidative damage.

The increase in the ILCM in hyperoxic fish acclimated to 25°C presumably decreases the functional lamellar surface area
area (not quantified in this study) for gas transfer. Previous data (41) showed that 7°C normoxic goldfish (ILCM present) do not have a significantly different arterial O2 than goldfish with protruding lamellae at 25°C, suggesting that the available functional surface area for O2 uptake in 7°C normoxic goldfish (~20% exposed lamellae) is sufficient to maintain arterial O2 at normal levels. Presumably, this is also true for 25°C goldfish with an ILCM given that these fish were also exposed to hyperoxia. It has also been shown that hyperoxia exposure of the closely related carp (Cyprinus carpio) at 25°C does not change the total blood oxygen content, which could also be true for hyperoxic goldfish at 25°C (39). This would suggest that the increase in the ILCM due to hyperoxia may be based on external cues rather than internal O2 content. The increase in ILCM may be beneficial because it decreases the ionoregulatory costs associated with replenishing salts lost by diffusion across the gill. Normally, such a morphological adjustment, while benefitting osmoregulation, would be expected to compromise respiratory function especially during hypoxia as demonstrated by an increase in critical O2 in crucian carp with covered lamellae (36) or sculpins exhibiting a thickening of the gill lamellae (12). However, our recent results suggest that respiratory function may, in fact, be unaltered during hypoxia by similar morphological changes in goldfish, perhaps owing to a rapid 20% loss of the ILCM as soon as hypoxia is encountered (37). This rapid loss may represent an emergency response to a sudden decrease in the ambient O2 levels (33).

Although there was a significant decrease in the ILCM during a temperature change from 7 to 25°C under hyperoxic conditions, the extent of this remodeling was less than when performed under conditions of normoxia. Thus, while hyperoxia in fish acclimated to 25°C was able to promote a gill phenotype that was indistinguishable from fish at 7°C, it could only reduce (and not totally prevent) the gill remodeling in fish transferred from 7 to 25°C. At the present time there is no clear explanation for this intermediate state of the ILCM after a temperature change under hyperoxic conditions. It is possible that there is a population of temperature sensors that also trigger gill remodeling independently, leading to a complex integrative system of temperature and O2 sensing that acts together to set the final gill phenotype.

**Perspectives and Significance**

The ability of fish to independently monitor the chemical status of both the external and internal environments is critical for their survival. The capability to rapidly detect potentially subtle changes in the ambient environment is important in triggering equally rapid compensatory responses aimed at minimizing any corresponding internal disturbances. Because changes in the internal chemical composition may also occur in the absence of any external perturbation, it is equally important to possess a sensory system able to detect fluctuations in the internal compartments. In this study, we provide direct evidence linking the O2 status of the internal environment to gill remodeling that can occur in the absence of any external cues. Because the internal trigger appeared to be hypoxemia, it supports the long-held view that fish possess receptors able to somehow sense the levels of total O2 content in the blood. Thus, it would seem that at least three key physiological processes: control of breathing (31, 35), the acute adrenergic stress response (27), and gill remodeling (present study) are controlled, at least in part, by receptors monitoring the blood O2 content. The notion of a sensor responding to changes in O2 content is not meant to exclude the possible contribution of lowered arterial or external O2 in themselves, as triggers for gill remodeling. The involvement of these cues was not specifically addressed in the present study. The results of the NaCN experiments provided evidence that activation of the NECs of the gill can promote gill remodeling. Because the NECs in other species (4, 16, 30) are known to respond specifically to lowered O2, and given their proximity to blood and water, it suggests that the remodeling process also can be regulated independently by changes in external or blood O2. Thus, it would appear that the gill remodeling process in goldfish is controlled by multiple factors that ultimately serve to link O2 uptake requirements to functional lamellar surface area.

**ACKNOWLEDGMENTS**

We are grateful to Bill Fletcher for his dedicated care of animals and Andrew Ochalski for help with microscopy.

**GRANTS**

This study was supported by Natural Sciences and Engineering Research Council (NSERC) Discovery and NSERC Research Tools and Innovation Grants to S. F. Perry.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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