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Hypoxia Tolerance in the Epaulette Shark (*Hemiscyllium ocellatum*)

GRAHAM WISE, JAMIN M. MULVEY, AND GILLIAN M.C. RENSHAW*
Vertebrate Neurobiology and Cell Biology Laboratory, Department of Anatomical Sciences, The University of Queensland, St. Lucia 4072, Queensland, Australia

ABSTRACT The epaulette shark, *Hemiscyllium ocellatum,* is a tropical reef shark that can live in an environment with cyclic periods of low oxygen concentration, suggesting that it has a well-developed capacity for anaerobic metabolism. Most investigations of hypoxia-tolerant teleosts and reptiles have focused on species that inhabit cold environments. This study was carried out on a tropical reef shark in order to determine whether similar strategies for hypoxia survival are used at higher environmental temperatures. We studied the effects of a single exposure to mild hypoxia and cyclic exposure to extreme hypoxia on blood-lactate concentration and key indicators of neurological function. The basal blood-lactate concentration for the epaulette shark was determined as 0.37 mM and showed a graded increase during hypoxia. After a single exposure to mild hypoxia (20% of normoxia for 4 h), the mean blood-lactate level rose to 3.07 mM ($P < 0.01$). After cyclic exposure to extreme hypoxia (eight repetitions of a 120-min exposure at 5% of normoxia), there was a rise in mean blood-lactate concentration to 5.43 mM ($P < 0.0001$). During both hypoxic regimens, there were no observed changes in key indicators of neurological function. We conclude that the epaulette shark is tolerant to both mild hypoxia and to cyclic exposure to extreme hypoxia. *J. Exp. Zool. 281:1–5, 1998.*

The epaulette shark, *Hemiscyllium ocellatum* (Bonnaterre, 1877), is an Australasian benthic reef shark that inhabits the shallow reef flats surrounding the islands of the Great Barrier Reef and the Torres Strait (Dingerkus and DeFino, '83; Last and Stevens, '94). This shark was studied at Heron Island, which is located in the Capricorn Bunker group of islands, on the Great Barrier Reef, situated off the northeastern coast of Australia. During a low tide at night, the water on the reef flat immediately surrounding the island does not mix with the surrounding ocean. Consequently, the respiration of coral, algae, and reef animals can lower the oxygen concentration from the normoxic level of 6.8 mg O_2/L to a hypoxic level of 2.1 mg O_2/L (Kinsey and Kinsey, '67). The survival of the epaulette shark on the reef flat in a naturally cycling hypoxic environment indicates that this shark may have a large capacity for anaerobic metabolism to avoid hypoxic damage.

The metabolic and neurological responses of teleost fish to hypoxia and anoxia have been examined in the hypoxia-susceptible rainbow trout (*Oncorhynchus mykiss*) and in the hypoxia-tolerant crucian carp (*Carassius carassius*). The brain of rainbow trout loses its neuronal ion-gradients after 30 min of anoxia (Nilsson et al., '93), whereas the crucian carp can survive anoxia for several days (Nilsson et al., '91). The differences between tolerant and nontolerant species are in part due to the increased capacity for anaerobic metabolism of tolerant species.

Most investigations into the hypoxia-tolerance of teleost fishes and turtles have focused on species that inhabit cold environments. The crucian carp can survive hypoxic periods of up to 5.5 months in ice-locked lakes (Blazka, '58). Similarly, the freshwater turtle, *Chrysemys picta bellii,* overwinters beneath frozen water-courses for up to 3.5 months (Ultsch, '89). However, hypoxia tolerance is reduced with increasing temperature (Herbert and Jackson, '85a,b; Kam and Lillywhite, '94). Many tolerant species show a preference for lower temperatures when they are placed in a hypoxic environment (Branco et al., '93; O'Connor et al., '88). The survival of the epaulette shark in a hypoxic tropical environment indicates that it may provide a useful model for examining the physiological mechanisms underlying hypoxia-tolerance.

*Correspondence to: Dr. Gillian Renshaw, Vertebrate Neurobiology and Cell Biology Laboratory, Department of Anatomical Sciences, The University of Queensland, St. Lucia 4072, QLD, Australia. E-mail: g.renshaw@mailbox.uq.edu.au
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We have examined whether the epaulette shark can survive two hypoxic regimens at 25°C without developing the neurological deficits that accompany hypoxic damage. In the first regimen, we tested cyclic exposure to extreme hypoxia; the level of oxyen concentration was at the extreme lower range conducted during previous investigations of hypoxia tolerance (Schurmann and Steffensen, '92; Dalla-Via et al., '94; van den Thillart et al., '94; Yoshikawa et al., '95). The second regimen employed mild acute exposure; oxygen concentration was comparable to that in the natural environment of the epaulette shark.

While most animals have a packed cell volume of 0.4 L/L (Schalm, '86), the epaulette shark has a packed cell volume of 0.14 L/L (M.H. Routley and G.M.C. Renshaw, unpub. results). Therefore, in order to avoid artifacts caused by collecting multiple samples each day, only the initial and final blood-lactate concentrations were measured to determine whether there had been a significant increase in anaerobic metabolism in response to hypoxia. The change in mean blood-lactate concentration after the mild acute hypoxic regimen was compared with the change in mean blood lactate after the severe chronic regimen to determine whether there was a graded anaerobic response, relative to each of the hypoxic regimens tested.

MATERIALS AND METHODS

Animal collection

Thirty adult male and nonpregnant female epaulette sharks were caught at low tide from the reef platform surrounding Heron Island. The sharks were 600 mm to 700 mm in length and were identified by recording individual patterns of spots between the ocellus on each side of the head. The animals were kept in a large flow-through saltwater pool for 24 h before the initial experiment and for the time intervals between experiments.

Experimental protocol

Two experimental regimens were used to investigate the effects of hypoxia on blood-lactate levels in epaulette sharks. The first experimental regimen examined the effect of cyclic exposure to extreme hypoxia. Twenty-two animals were assigned to control and experimental groups so that the length and sex ratio of the animals in each group were similar. In each of four replicate experiments, three untreated and three experimental animals were placed in separate but identical tanks, each containing 100 L of seawater at 25 ± 1°C and normal oxygen saturation (6.8 mg O_2/L). The water was continuously circulated within each tank throughout the experiment. Untreated animals were maintained at normoxia in aerated seawater. The experimental group was maintained in a hypoxic environment by bubbling nitrogen gas through the water at a rate of 4 L/min to displace the dissolved oxygen over a 20-min period. Thereafter, the flow of nitrogen and air was regulated to maintain the oxygen concentration at 0.39 mg O_2/L (5.7 ± 0.05% normoxia) for a further 100 min. The experimental tanks were fitted with clear perspex lids to prevent oxygen re-equilibration during the experiment. This regimen was repeated twice a day at 10-h intervals for 4 days.

The second experimental regimen examined a single exposure to mild hypoxia, similar in magnitude to that experienced by epaulette sharks in their natural environment. Two groups of three sharks were placed in the experimental tanks described above and were exposed to 1.55 ± 0.04 mg O_2/L (20% ± 0.5% normoxia) for 240 min. Oxygen levels were measured and temperature was recorded in each tank using a YSI oxygen meter and probe. The oxygen meter was calibrated in humidified air. The normal oxygen saturation of seawater at 25°C was calculated to be 6.8 mg O_2/L (88% of the oxygen saturation in humidified air).

Analysis of blood-lactate concentration

Blood samples were collected rapidly from each shark by caudal venipuncture before the animal commenced struggling. The first sampling was taken before the first exposure to hypoxia or normoxia, and the second sample was taken at the end of the final experiment so that the final change in blood-lactate level could be calculated for each shark. The blood samples were collected into EDTA-fluoride blood collection tubes and spun in a centrifuge at 2000*g* for 10 min. The plasma was collected and stored at –70°C. Analysis of blood-lactate concentration was carried out on a Cobas Mira automatic analyser using an enzymatic lactate analysis kit (Boehringer-Mannheim, Australia Pty Ltd).

Neurological assessment

In a parallel experiment, three sharks were placed in the experimental tank and exposed to a dissolved-oxygen concentration of 0.39 mg O_2/L (5.7% of normoxia). Sharks were handled at 15-min intervals for 120 min to monitor their neurological responses. Five parameters were measured:

(1) righting reflex; (2) responses to external stimuli; (3) rhythmic gill movements; (4) patterned motor output required to generate a swim response (rhythmic swimming); and (5) movement of diagonally opposed fins for locomotion. For the remaining 30 sharks, observations were made through the Plexiglass lid during the experiments, and the neurological assessment took place when animals were returned to the holding pool at the conclusion of each session in the tank.

Statistical analysis

Within-group differences in variance were tested using two-tailed paired Student's *t*-tests. Between-group differences in variance were tested using two-tailed unpaired *t*-tests. Values are presented as the mean ± SEM.

RESULTS

Oxygen displacement and blood-lactate levels

The time course for dissolved oxygen in the experimental tank followed an exponential decrease. The mean baseline lactate concentration for all sharks was 0.37 ± 0.06 mM (Figs. 1a, 1b, and 2). After cyclic exposure to extreme hypoxia, the mean blood-lactate concentration in experimental animals rose significantly from 0.47 ± 0.13 to 5.43 ± 0.47 mM ($P < 0.0001$; Fig. 1a).

In the untreated confined group, maintained at normal oxygen saturation, the mean blood-lactate level rose from 0.24 ± 0.04 to 0.62 ± 0.12 mM. This small increase was significant ($P < 0.05$) and reveals that confinement can increase blood-lactate concentration (Fig. 1b). While the initial blood-lactate concentration was not significantly different between the untreated and experimental groups, the final mean blood-lactate concentration of the animals exposed to severe chronic hypoxia differed significantly ($P < 0.0001$), showing that the increase in lactate concentration measured in the experimental group was significantly different than caused by confinement alone (Fig. 1a,b).

After a single exposure to mild hypoxia, the mean blood-lactate level rose from 0.40 ± 0.05 to 3.07 ± 0.58 mM. This 7.7-fold increase was significant ($P < 0.01$) and was higher than expected from the effects of confinement alone (Fig. 2).

Neurological assessment

Throughout the experiment, no alterations to the key indicators of neurological function were observed. All of the animals were able to right themselves, move away from light pressure on

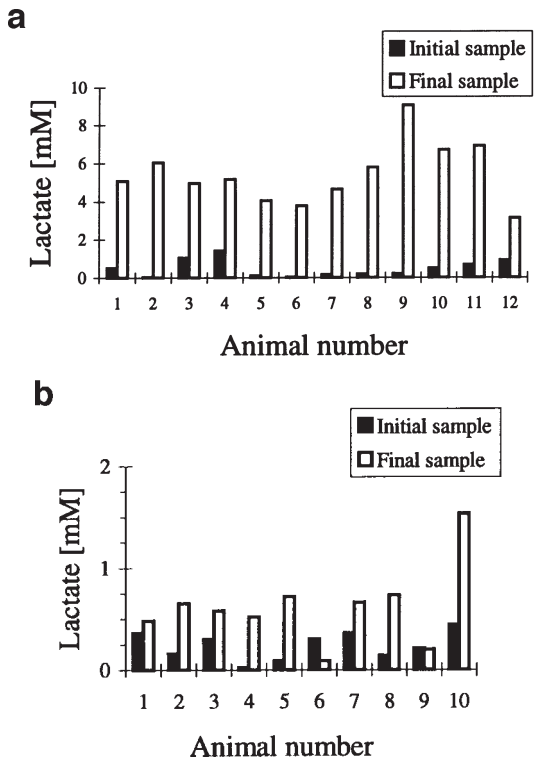

Fig. 1. (**a**) Blood-lactate concentration for individual experimental animals before and after cyclic exposure to extreme hypoxia. The initial sample was taken at the beginning of the experiment (6.80 mg O_2/L), and the final sample was taken after eight exposures, at 10 hourly intervals, to extreme hypoxia in a 100-L tank (0.39 mg O_2/L). (**b**) Blood-lactate concentration for individual control animals. The initial sample was taken at the beginning of the experiment (6.80 mg O_2/L), and the final sample was taken after eight exposures, at 10 hourly intervals, to normoxia in a 100-L tank (6.80 mg O_2/L).

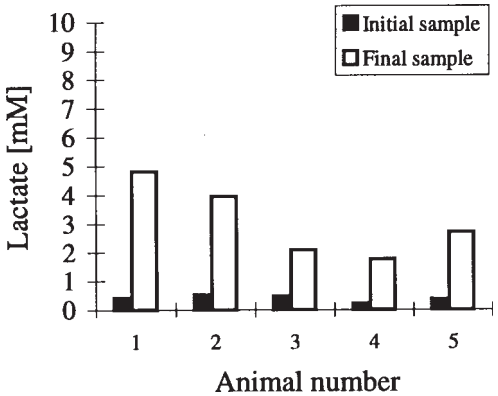

Fig. 2. Blood-lactate concentration for individual experimental animals before and after acute exposure to mild hypoxia. The initial sample was taken at the beginning of the experiment (6.80 mg O_2/L), and the final sample was taken after a single exposure to mild hypoxia in a 100-L tank (1.55 mg O_2/L).

the pectoral fin, maintain rhythmic gill movements, engage in rhythmic swimming, and use diagonally opposed fins for locomotion. Both experimental and untreated animals maintained voluntary movement, normal body tone, and a characteristic posture while in the tanks. Rhythmic swimming and the righting reflex were always maintained when the sharks were returned to the holding pool. These data indicate that neither of the hypoxic regimens produced neurological deficits during or after the rise in blood-lactate concentration.

During an earlier series of pilot experiments carried out at 0.39 mg O_2/L for over 3.5 h, no impairment of neurological function occurred until after 3.5 h. The righting response was temporarily impaired after 4.0 h of hypoxia but upon re-oxygenation the righting reflex returned within 30 min. No permanent impairment to any of the neurological indicators was evident at 24 h post-experiment.

DISCUSSION

Baseline blood lactate concentration

The mean blood-lactate concentration for the epaulette shark was 0.37 ± 0.06 mM. This value is comparable to the blood-lactate levels reported for benthic elasmobranchs. Blood-lactate concentrations ranging from 0.59 to 0.63 mM have been reported for the spiny dogfish, *Squalus acanthius* (DeRoos et al., '85; DeRoos and DeRoos, '92). However, studies of more active elasmobranchs have reported higher baseline lactate concentrations. The blood-lactate level for the leopard shark, *Triakis semifasciata,* was reported to be 1.79 mM, which is more than double that reported for the spiny dogfish (Lai et al., '90).

Increased blood lactate concentration in response to hypoxia

An increase in lactate concentration is indicative of an increase in anaerobic metabolism in the elasmobranch *Torpedo marmorata* (Hughes and Johnston, '78). Significant elevations in blood-lactate concentration occurred in response to hypoxia in the epaulette. The blood-lactate level rose 7.7-fold after a single exposure to mild hypoxia (1.55 mg O_2/L) and increased 11.6-fold after repeated exposure to extreme hypoxia (0.39 mg O_2/L). The final blood-lactate level returned to baseline level within 10 h (Routley and Renshaw, unpub. data), so it is likely that the final blood lactate in this study reflected the lactate level after each hypoxic exposure. It is possible that hypoxic preconditioning occurs in these animals, and this possibility is being investigated currently. These data indicate that the epaulette shark has a graded response to hypoxia that involves a significant increase in the level of anaerobic metabolism. The increase in lactate would be transient because, upon re-oxygenation, lactate is used as an oxidative fuel and a gluconeogenic substrate in elasmobranchs (Phillip and Hird, '77; Bennett, '78; Coulson, '79; DeRoos et al., '85).

The blood-lactate data from a single exposure to mild hypoxia indicate that an increased blood-lactate concentration occurs at a concentration of dissolved oxygen likely to be encountered by the epaulette shark in its natural environment. Consequently, on the reef flat, blood-lactate concentration in the epaulette shark may follow a lunar cycle, with its highest peak occurring during nocturnal low tides. The data from the cyclic exposures to extreme hypoxia demonstrate that the level of blood lactate in the epaulette shark increased in relation to the severity of hypoxia encountered. However, the data also indicate that a proportion of the elevation in blood lactate may be due to confinement.

Neurological status during and after hypoxia

During the acute, chronic, and untreated regimens, there were no observed changes in the five neurological parameters examined in this study. In addition, the sharks maintained their characteristic body posture and voluntary movements throughout the experiments. These findings contrast with studies of hypoxia-intolerant species, such as the rainbow trout, which suffers a rapid loss of equilibrium in response to hypoxia (Yamamoto and Lida, '94).

The maintenance of voluntary activity of the epaulette shark during exposure to severe hypoxia (0.39 mg O_2/L) also contrasts with the activity of freshwater turtles and amphibians during hypoxia. When freshwater turtles are exposed to hypoxia or anoxia in their natural environment, they also encounter low water temperatures and enter a hibernating state and cease feeding; this phase is followed by torpor and loss of voluntary movement (Sexton, '59; Gibbons, '68). Hypoxia-tolerant amphibians decrease their respiratory rate, cease spontaneous movement, lose body posture, lose their righting reflex, and finally become paralysed (Moratzky et al., '93).

The differences between the response of the epaulette shark and other hypoxia-tolerant vertebrates studied may reflect differences in the natural environment of these animals. Some freshwater turtles and crucian carp experience low oxygen concentration in their environment at low or near-freezing temperatures. However, the epaulette shark experiences low oxygen concentration in its environment at times when the water temperature ranges from 23°C–25°C. In addition, we observed that the epaulette shark is still active during nocturnal low tides when the oxygen concentration is about 2.1 mg O_2/L (30% of normoxia) on the reef platform. In summary, in this study we have demonstrated that, at tropical water temperatures, the epaulette shark is hypoxia-tolerant at levels of oxygen concentration well below those encountered in its natural environment.

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