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A POSSIBLE ROLE FOR CATECHOLAMINES IN THE VENTILATORY RESPONSES ASSOCIATED WITH INTERNAL ACIDOSIS OR EXTERNAL HYPOXIA IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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Summary

Plasma catecholamine levels and gill ventilation were measured in rainbow trout (*Oncorhynchus mykiss*) during acidosis and hypoxia. There was an increase in both plasma catecholamines and ventilation correlated with the acidosis. Fish exposed to hyperoxia prior to acid infusion did not show significant changes in catecholamines or ventilation. Those treated with the β-adrenergic antagonist propranolol before acidosis showed increases in catecholamines but not ventilation. Hypoxia was also associated with increases in endogenous catecholamines and ventilation, and the increase in ventilation could be partially blocked with propranolol. This increase in ventilation during hypoxia was not inhibited by a saline injection alone. It is proposed that catecholamines act to modulate ventilatory responses in fish under both acidotic and hypoxic conditions. If a central H^+ chemoreceptor exists in fish to control breathing, it can be inhibited by hyperoxia or by β-receptor blockade.

Introduction

The catecholamines noradrenaline (NOR) and adrenaline (ADR) are released into the blood of fish by stress, including hypoxia (Randall, 1982) and acidosis (Primmett *et al.* 1986; Boutilier *et al.* 1988), as well as other conditions such as exercise (Primmett *et al.* 1986) and physical disturbance (Nakano and Tomlinson, 1967). Catecholamines can exert a wide variety of effects on the physiology of fish, for example, on the distribution of red blood cells (Holbert *et al.* 1979), on the permeability of the gill epithelium (Isaia *et al.* 1978), on oxygen transport (Nikinmaa, 1982*b*) on red blood cell volume and intracellular pH (Nikinmaa,

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1982*a*) and on the branchial vascular resistance to flow (Wood, 1975). Increases in ventilation have also been observed when fish have been exposed to stressful situations (Hughes and Saunders, 1979; Randall and Jones, 1973). In contrast, Smith and Jones (1982) found no increase in ventilation under hyperoxic hypercapnic conditions, although catecholamine levels were not monitored in this case. Peyraud-Waitzenegger (1979) also observed that infusion of catecholamines caused increased ventilation in eels during the summer, and that the response could be blocked by a β-adrenoceptor antagonist, propranolol. After β-blockade, she noted an α-adrenoceptor-mediated hypoventilatory response to adrenaline similar to that seen in winter when the animals show only an α effect. In addition, injection of noradrenaline or adrenaline in rainbow trout is correlated with an increase in ventilation (S. Aota, unpublished observations). Therefore, the first part of the present study was designed to look at the possible role of catecholamines in ventilatory responses to a stress (acidosis) in rainbow trout in order to determine if ventilatory adjustments are dependent on the release of catecholamines.

Hypoxia causes increases in ventilation and increases in circulating catecholamines (Holeton and Randall, 1967; Hughes and Saunders, 1970; Smith and Jones, 1982; Boutilier *et al.* 1988). It has been postulated that reduced blood O_2 content stimulates some chemoreceptor, leading to an increase in ventilation. Another possibility is that hypoxia causes catecholamine release, which in turn stimulates ventilation. The second part of this study was designed to determine whether increases in ventilation during hypoxia were due to the release of endogenous, circulating catecholamines and their subsequent stimulation of the respiratory centre.

Materials and methods

Experimental animals

Rainbow trout (*Oncorhynchus mykiss*, 245–499 g) from a local hatchery (West Creek Trout Ponds, Aldergrove, BC) were held outdoors in a large fibreglass tank. They were fed commercial trout pellets once a week, and were not fed for at least 48 h before surgery. Aerated, dechlorinated Vancouver tapwater flowed through the tank, the temperature ranging from 8 to 17 °C. The temperature of the water used in each experiment was the same as that in the holding tanks. Temperature varied by ±0.5 °C during each experiment.

Surgical procedures

All surgery was performed under general anaesthesia. After catching the fish, it was anaesthetized in a 1:10 000 solution of tricaine methanesulphonate, MS-222 (Syndel Laboratories, Vancouver, BC), buffered to pH 7.5 with sodium bicarbonate, then weighed. Next, the fish was transferred to an operating table similar to the one used by Smith and Bell (1967). During surgery, the gills were irrigated with a less concentrated anaesthetic solution (1:20 000 MS-222). Every fish had its dorsal aorta cannulated, as described by Soivio *et al.* (1975), with polyethylene

tubing (PE-50, Intramedic, Parsippany, NJ). This cannula was used for blood sampling and injection purposes. For the HCl injection series, a second cannula (PE-190) was placed through the snout into the buccal cavity, so that the flared tip was resting against the roof of the mouth. This second cannula was used to measure ventilation parameters. The fish recovered in darkened Perspex boxes for 48 h. In the hypoxia exposure series, each trout had an oral membrane sutured around the mouth, following the technique of Cameron and Davis (1970). The fish was then allowed to recover in a van Dam chamber with a positive pressure head for 36 h. During the recovery periods, the cannula was flushed with 0.15 ml of heparinized (10 i.u. ml^{-1} heparin) Cortland's saline (Wolf, 1963) once every 12 h.

Experimental protocol

Series I: the effect of acid infusion on gill ventilation

Experimental treatment. All treatments involved the creation of blood acidosis through a bolus injection of a 5 ml kg^{-1} body mass solution of HCl (Fisher Scientific, Pittsburg, PA) in Cortland's saline. The acid concentration of the injected solution was 0.04 mol l^{-1}, with infusion times of 4–8 min. The animals were subjected to one of three conditions. The first condition was acid infusion alone. The second involved acid infusion following a 30 min exposure to oxygen bubbled into the anterior of the Perspex box, making the blood hyperoxic. In the third condition, the acid infusion was preceded by a 30 min exposure to a 1 ml injection of 2.5×10^{-4} mol l^{-1} DL-propranolol HCl (Sigmal Chemical Co., St Louis, MO) in saline. Propranolol is a β-blocker, and was used to block any β-adrenoceptor-mediated effects of circulating catecholamines on ventilation (Peyraud-Waitzenegger, 1979).

Blood samples. Two blood samples of 1.0 ml each were removed from the dorsal aortic cannula, and were replaced by an identical volume of heparinized saline. The first sample was taken just prior to the injection of the hydrochloric acid solution (pre-treatment), i.e. 30 min after the exposure to hyperoxia or injection of propranolol; and the second was obtained 5 min after the acid injection had been completed. For each 1.0 ml sample, 500 μl was used to measure blood pH and blood oxygen tension (Pa_{O_2}). The balance of the blood was centrifuged and the plasma removed and frozen in liquid nitrogen. The frozen plasma was stored in a freezer at −80 °C until later analysis for catecholamines.

Ventilatory parameters. The buccal cannula was connected to a pressure transducer (Statham P23BB, AST/Servo Systems Inc., Newark, NJ) and a chart recorder (Gould Brush 260, Gould Inc., Instrument Systems Division, Cleveland, OH). Ventilation frequency (f_G) was counted as the number of peaks in a 1 min period in both the pre- and post-treatment areas of the trace. Buccal pressure (P_{bu}), which was used as a measure of ventilatory stroke volume, was taken as the average peak pressure (Pa) averaged over 10 cycles in the same 1 min interval. Control values were taken from the portion of the chart corresponding to 30 min following the onset of hyperoxia or propranolol injection, just prior to the acid injection.

Series II: the effect of external hypoxia on gill ventilation

Experimental treatment. Hypoxic conditions were created by passing water and nitrogen gas in countercurrent directions through an oxygen-stripping column. Inspired water oxygen tension ranged from 3.2 to 8.0 kPa (mean 6.3 kPa) after passing through the column. The first treatment was exposure to hypoxia alone. In the second condition, a propranolol injection (see HCl injection series above) was again used in an attempt to prevent the possible effects of circulating catecholamines on ventilation. The final treatment was the injection of 1 ml of heparinized saline 30 min prior to exposure to hypoxia. This was a control treatment to see if injection alone could block the ventilatory response to hypoxia. In all treatments, the exposure to hypoxia was maintained for 30 min.

Blood samples. Again, two blood samples of 1.0 ml each were taken from the trout and replaced with an equal volume of heparinized saline. The initial sample was taken immediately prior to the exposure to hypoxia and, for the propranolol- and saline-treated fish, 30 min after the injections. The blood samples were treated in the same manner as described above for the acid injection series, with one addition: 50 μl of whole blood was drawn off to determine haematocrit in a microhaematocrit tube (i.d. 1.1–1.2 mm, Fisher Scientific, Pittsburg, PA).

Ventilatory parameters. For this series, the ventilation measurements were made in a van Dam box. When the oral membrane had been fixed to the partition of the box, the box was split into two chambers, and the only path for the water to take from the anterior to the posterior chamber was through the mouth and gills. One hour before the experiments began, the overflow drains were set so that the water levels in the front and back chambers were at equal levels visually, and the oral membrane was slack. This indicated a zero pressure head, so that the fish would have to ventilate actively. All overflow from the rear chamber, in this situation, would be from water that the fish had ventilated through its mouth and gills. Ventilation frequency was recorded by the counting the number of mouth movements in 1 min, and minute ventilation volume (V_G) was measured by collecting the overflow of the rear chamber for 1 min. These values were recorded 1–3 min prior to blood sampling so that the sampling itself was not affecting them.

Analytical techniques

Measurement of whole-blood oxygen tension was made using a Radiometer Copenhagen E5046 P_{O_2} electrode in a D616 thermostatted cell and a PHA 930 P_{O_2} module in conjunction with a PHM 71 acid–base analyzer. The P_{O_2} electrode was calibrated using nitrogen gas and air-saturated water. Blood pH was measured using a Radiometer Copenhagen G297/G2 glass capillary electrode with a PHM 71 acid–base analyzer. Calibration of the pH electrode was made using Radiometer Copenhagen precision buffer solutions standards S1510 and S1500. Analyses of plasma catecholamine levels were performed by high pressure liquid chromatography (HPLC) with electrochemical detection, using a Brownlee Spheri-5 reverse-

Table 1. *Blood oxygen tension, acid–base status and gill ventilation in the three treatments of series I*

	Time (min)	HCl	Propranolol/ HCl	Hyperoxia/ HCl
Mass (g)		400±26	427±15	434±19
Pa_{O_2} (kPa)	0	14.3±0.7	17.2±0.4	50.3±1.5†
	5	17.7±2.0*	18.9±0.9*	52.7±0.8*
pHe	0	7.75±0.05	7.83±0.06	7.87±0.04
	5	7.49±0.06*	7.50±0.05*	7.51±0.05*
f_G (min^{-1})	0	74±4	78±2	78±3
	5	77±4	76±3	77±3

* A significant difference from control (0 min) values; † a significant difference from HCl-treated fish in the control or 5 min measurement.

All values are given as mean±standard error (S.E.); N=6 in all three treatments.

f_G, ventilation rate.

phase column (Technical Marketing, Richmond, BC), a Bioanalytical Systems LC-4A amperometric detector (Mandel Scientific, Rockwood, Ontario) and a Spectra-Physics SP 8700 solvent delivery system (Terochem Laboratories Ltd, Edmonton, Alberta), as described by Primmett *et al.* (1986) and Woodward (1982).

Statistical significance of data was determined by two-way analysis of variance (ANOVA), one-way ANOVA, Kruskal–Wallis one-way ANOVA or Student's paired *t*-test, as appropriate, with a statistical significance level of 5%.

Results

Series I: responses to blood acidosis

Blood pH and Pa_{O_2}

In all treatment groups, there was a significant drop in pH, from 7.82±0.03 to 7.51±0.04 (mean±standard error), a change of 0.31 pH units, in the 5 min following injection of the acid solution (Table 1). There was no significant pH difference between the initial control groups for the three treatment conditions, or between the post-injection pH values. There was a significant increase in arterial blood P_{O_2} (Table 1) under all three treatment groups, with an increase of 2.4 kPa in hyperoxia-treated fish and an increase of 1.7–3.5 kPa for the other two groups. The initial Pa_{O_2} for the hyperoxia/acid injection condition was significantly higher than the other two control values, but this was simply due to the prior exposure of the animal to hyperoxic water for 30 min.

Ventilatory responses

Infusion of acid alone was the only treatment condition where a significant ventilatory change was observed (Fig. 1) In this case, the average P_{bu} increased

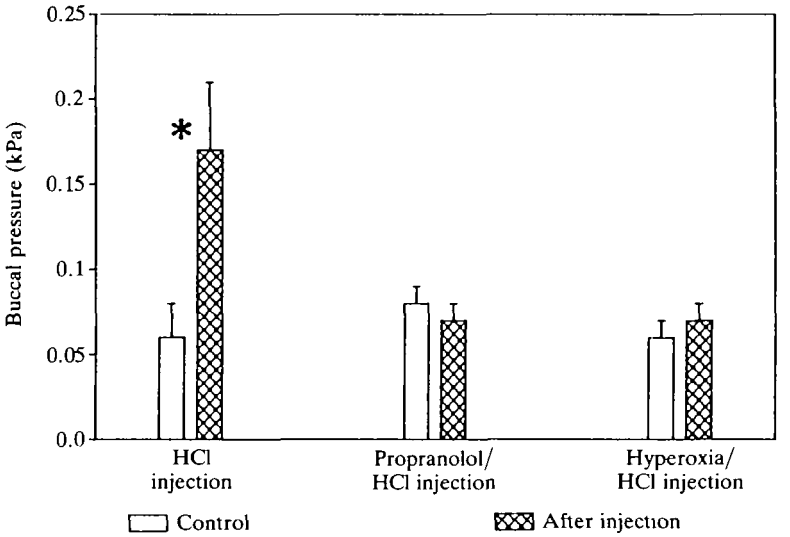

Fig. 1. Breathing changes in rainbow trout injected with $0.04\,mol\,l^{-1}$ HCl, injected with $2.5\times10^{-4}\,mol\,l^{-1}$ propranolol, prior to the HCl injection, and exposed to hyperoxia prior to the HCl injection. *indicates a significant difference in the post-injection value from control. $N=6$ in all three treatments. Values are presented as mean+S.E.

significantly from 0.06 to 0.17 kPa, a rise of 0.11 kPa. The other two treatments showed no change. There was no significant difference between control P_{bu} values across the group. f_G did not change significantly with any of the three treatments.

Catecholamine changes

In the experimental animals treated with either acid alone or acid after propranolol exposure, circulating catecholamines were present at significantly higher concentrations following acid injection than they were at rest (Fig. 2). After acid injection alone, adrenaline concentration increased by an average of $0.111\,nmol\,l^{-1}$, from $0.009\,nmol\,l^{-1}$ to $0.12\,nmol\,l^{-1}$, and noradrenaline concentration increased by an average of $0.132\,nmol\,l^{-1}$, from 0.008 to $0.14\,nmol\,l^{-1}$. Although the absolute level of change in plasma catecholamines is small, the post-experimental values are 13 times (ADR) and 18 times (NOR) greater than the control values. Acid injection into propranolol-treated fish also resulted in increases in both adrenaline (12 times) and noradrenaline (11 times) levels. The fish exposed to hyperoxia prior to the acid injection showed no significant increase in plasma catecholamines. Resting catecholamine levels between the treatments did not differ from each other, but the amounts of catecholamines released by the propranolol-treated trout were significantly higher than those released from fish treated with the acid injection only.

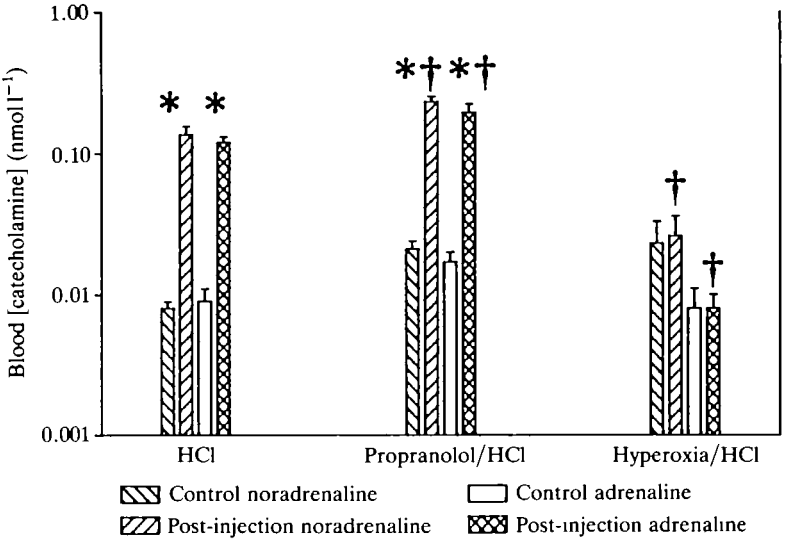

Fig. 2. Changes in blood catecholamine levels of trout injected with 0.04 mol l^{-1} HCl, injected with 2.5×10^{-4} mol l^{-1} propranolol prior to the HCl injection and exposed to hyperoxia prior to the HCl injection. * indicates a significant difference in the post-injection value from control. † indicates a significant difference from the HCl-treated fish in the control or post-injection measurement. $N=6$ in all three treatments. Values are presented as mean+S.E.

Series II: responses to external hypoxia

Blood pH, Pa_{O_2} and haematocrit

Under all three conditions, there was a significant drop in Pa_{O_2} (Table 2). Control values between all treatments were not significantly different, but the post-treatment value for the saline-treated fish was significantly higher than those for the other two treatments. A significant plasma acidosis (Table 2) was also observed in all experimental animals, with a mean drop of 0.18 pH units. Blood haematocrit showed a small (0.5 %) but insignificant increase (Table 2). Blood pH and haematocrit showed no significant differences between the three treatments for the control or hypoxic values.

Ventilatory responses

All treatments showed significant increases in ventilation volume (Fig. 3). The increases seen in the untreated and saline-treated fish were not significantly different from each other but were significantly higher than the increase in the propranolol-treated fish. Control values were not significantly different from each other. The differences between the control and hypoxic breathing rates (Table 2) were not significant in all three treatments, but the control breathing rate of the saline-treated fish was significantly lower than those of the other two groups.

Table 2. *Blood oxygen tension, acid–base status and gill ventilation in the three treatments of series II*

	Time (min)	Hypoxia (N=7)	Propranolol/ hypoxia (N=10)	Saline/ hypoxia (N=5)
Mass (g)		293±11	311±24	335±10
Pa_{O_2} (kPa)	0	9.9±2.3	10.0±1.1	13.9±0.5†
	30	1.7±0.4*	1.5±0.3*	3.7±0.4*†
pHe	0	7.90±0.04	7.82±0.04	7.87±0.09
	30	7.67±0.08*	7.61±0.09*	7.82±0.09*
Haematocrit	0	27.8±3.1	28.7±2.6	30.0±3.2
	30	28.1±4.8	25.8±4.8	31.7±3.4
f_G (min)	0	100±4	93±5	80±4†
	30	90±13	85±11	81±4

* A significant difference from control (0 min) values; † a significant difference from hypoxia-treated fish in the control or 30 min measurement.

All values are given as mean±standard error (S.E.).

f_V, ventilation rate.

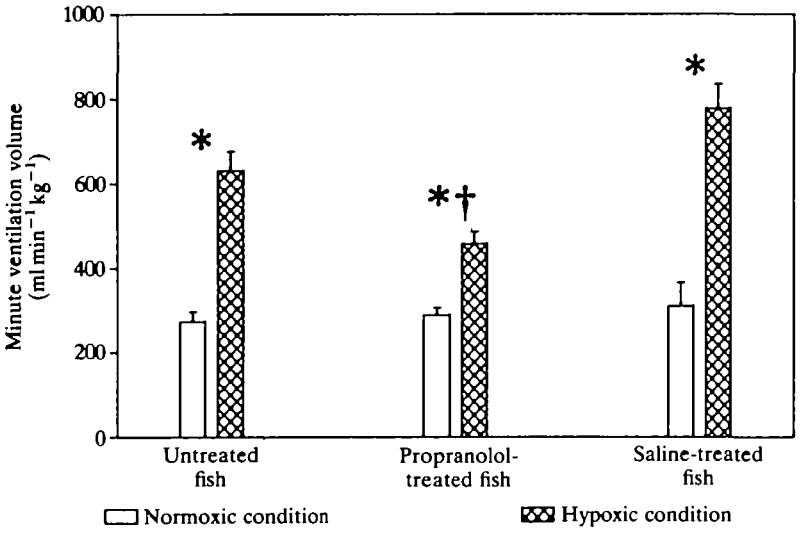

Fig. 3. The effect of hypoxia on the minute ventilation volume of untreated rainbow trout (N=7), fish pre-treated with an injection of 2.5×10^{-4} mol l^{-1} propranolol (N=10) and trout pre-treated with a sham injection of saline (N=5). * indicates a significant increase of the hypoxic value from the normoxic control. † indicates a significant difference from the untreated fish in the control or hypoxic measurement. Values are presented as a mean+S.E.

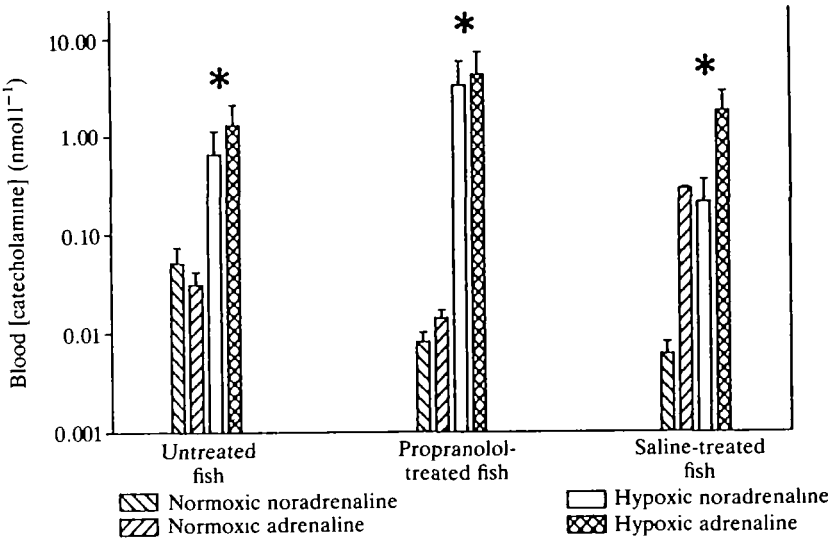

Fig. 4. Alteration of blood catecholamine levels during hypoxia in untreated rainbow trout (N=7), fish pre-treated with an injection of 2.5×10^{-4} mol l^{-1} propranolol (N=10) and fish pre-treated with a sham injection of saline (N=5). * indicates a significant increase of the hypoxic value from the normoxic control. Values are presented as mean+S.E.

Catecholamine changes

Both noradrenaline and adrenaline levels (Fig. 4) showed highly significant increases during hypoxia exposure in all three treatments. For noradrenaline, there was an overall change from 0.022 to 1.75 nmol l^{-1}, an increase of 80-fold. Adrenaline levels increased more, 120-fold, from 0.024 to 2.87 nmol l^{-1} (Fig. 4). There were no significant differences between the three treatments for either catecholamine.

Discussion

Series I: modification of ventilation to blood acidosis

Under all three treatment conditions, infusion of HCl was reflected by a significantly reduced plasma pH. This induced acidotic state was associated with a ventilatory increase in the group of fish treated by acid infusion alone. This would indicate the existence of a central chemoreceptor, sensitive to hydrogen ions, that controls ventilation, as is found in air-breathers (for a review, see Shelton *et al.* 1986). However, since no change in ventilation was observed in either hyperoxic or propranolol-treated fish upon acid infusion, either there is an H^+ receptor present that can be inhibited by β-receptor blockade or by hyperoxia, or no central H^+ receptor exists in fish to control ventilation and the response is mediated by another mechanism.

It has been demonstrated that the hyperventilation observed in eels caused by adrenaline infusion can be inhibited by β-adrenoceptor blocking agents (Peyraud-

Waitzenegger, 1979). Catecholamines are released in response to acid infusion, and it is possible that they act to mediate the increase in ventilation in rainbow trout exposed to acidotic conditions. The ventilatory increases observed in fish treated with acid alone were not seen when there was no significant catecholamine release (hyperoxia), or when the adrenaline and noradrenaline levels increased and β-receptors were blocked with propranolol. These results imply that catecholamines can act to mediate increases in ventilation during acidotic conditions in rainbow trout.

Catecholamines enhance oxygen transfer in fish by many means, including alterations in gill water flow, blood flow and blood oxygen transport (Perry *et al.* 1989). The presence or absence of the catecholamines can explain the increases in the arterial blood oxygen tension observed in all three treatments, although the reasons for the increases are different in each case.

The oxygen partial pressure increase in fish infused with acid alone is attributable to a combination of the observed increase in ventilation and the expected increases in gill perfusion (Farrell *et al.* 1980) and gill permeability (Isaia *et al.* 1978) induced by adrenaline and noradrenaline release. Whether oxygen off-loading of the red cells also contributed to the observed increase in Pa_{O_2} is not clear, for, although the presence of catecholamines should prevent the Bohr and Root shifts (Nikinmaa, 1982*a*), eliminating the possibility of Pa_{O_2} increases *via* this effect, the extent of catecholamine mobilization may not have been sufficient to prevent O_2 off-loading.

The change in Pa_{O_2} in acid-infused hyperoxic fish was probably due to a Bohr shift decreasing haemoglobin oxygen-binding, since catecholamines were not released. Erythrocyte acidification would essentially result in a decrease in the amount of haemoglobin available for binding, the response being reflected in an overall Pa_{O_2} increase. Although the initial Pa_{O_2} was relatively high in the hyperoxic fish, owing to the oxygen exposure prior to acid infusion, the magnitude of the increase following acid infusion was no different from the values reported for the other two treatment groups (Table 1).

Since ventilation did not change with acid infusion in propranolol-treated fish, the Pa_{O_2} increases cannot be ascribed to increases in breathing, even though catecholamine levels went up. The catecholamine release may have caused some haemodynamic changes in the gills, resulting in a slight Pa_{O_2} increase but, without an associated hyperventilatory response, it is unlikely that a change of the same magnitude as in the other two groups would have been observed. Assuming that the effects of catecholamines on red blood cells are mediated by β-receptors on the cell membrane (Nikinmaa, 1982*a*; Primmett *et al.* 1986), propranolol would have blocked adrenaline and noradrenaline effects, thus allowing Bohr and Root shifts to occur. This could account for the observed plasma Pa_{O_2} increase in the same manner as was seen in the hyperoxic animals.

It is also possible that the responses to the acid injection are to a high partial pressure of CO_2 (P_{CO_2}) rather than to low pH, for one would expect titration of the bicarbonate in the saline when the acid was added to it, resulting in a high P_{CO_2},

and it was not possible to differentiate between a response to acidosis and a response to high P_{CO_2}. However, the solution was exposed to the atmosphere for some time before injection into the trout (about 3–5 min), and so the P_{CO_2} of the solution would have equilibrated with the atmospheric P_{CO_2} (H. Lin, personal communication). If the animal was responding to P_{CO_2}, it responded only when the catecholamines could have an effect, i.e. there was no response when catecholamines were not released or when the β-adrenoceptors were blocked with propranolol.

Series II: modification of ventilation in response to external hypoxia

There were increases in noradrenaline and adrenaline levels in all treatments involving hypoxia. Elevated plasma catecholamine levels during hypoxia have been observed in the dogfish *Scyliorhinus canicula* (Butler *et al.* 1978) and in the rainbow trout *Oncorhynchus mykiss* (Boutilier *et al.* 1988). Water flow over the gills increases during hypoxia. Differences between normoxic and hypoxic breathing rates were not significant, and increases in gill water flow were achieved by increases in breathing amplitude. Most teleosts increase breathing amplitude rather than ventilation frequency during stress (Shelton *et al.* 1986), including *Oncorhynchus mykiss* (Davis and Cameron, 1971; Randall and Jones, 1973; Iwama *et al.* 1987).

It has been hypothesized that increased levels of circulating catecholamines mediate the increased ventilatory responses to hypoxia. This hypothesis was not completely disproved by our experiments. Figs 3 and 4 show that there is a definite increase in NOR, ADR and V_G during hypoxia. They also show that propranolol can partially block the ventilatory response of fish to hypoxia. This partial blockade was due to the propranolol, and was not an artefact of the injection itself, because hypoxia caused a definite ventilatory increase after an injection of saline alone. It is obvious from our results that catecholamines can modify the ventilatory pattern seen during hypoxia in these fish. As only a partial blockade was seen, the other component causing an increase in ventilation is most likely to be derived from a direct chemoreceptor-driven response to hypoxia. It would appear that, during severe hypoxia, catecholamines act to potentiate the ventilatory response of the animal. It has recently been shown that catecholamines are not involved in the ventilatory responses of the fish to mild hypoxia (R. Kinkead and S. F. Perry, in preparation); however, a difference between that study and the present study is in the level of hypoxia presented to the animals, 9.6 *versus* 6.3 kPa. It is probable that when the trout are exposed to a moderate level of hypoxia, such as 9.6 kPa, there is no catecholamine release and the oxygen chemoreceptor mediates all increases in V_G. However, when the chemoreceptors in trout are exposed to an external oxygen tension of less than 6.7 kPa, chemoreceptor activity is depressed (M. L. Burleson, personal communication) and, at the same time, blood catecholamines increase. We postulate that, in this situation, catecholamines are acting to mediate the increase in ventilation during hypoxia.

A possible mechanism for this is that circulating catecholamines cross the

blood–brain barrier and stimulate adrenergic receptors, which, in turn, cause a stimulation of the respiratory centre, and the output is seen as an increase in V_G. The β-blocker prevents the stimulation of the adrenoceptors, so that the increase in V_G is reduced. Of the two catecholamines, noradrenaline is more likely to be the catecholamine involved as it is able to pass through the blood–brain barrier more easily than adrenaline (Nekvasil and Olson, 1986).

The above mechanism would represent the efferent arm of the animal's response to severe hypoxia. The afferent arm depends upon the development of a hypoxic state leading to a release of plasma catecholamines. In teleost fish, chromaffin tissues (the source of circulating catecholamines) of the head kidney and posterior cardinal vein are highly innervated by cholinergic nerves (Hathaway *et al.* 1989) and release catecholamines in response to hypoxaemia (Perry *et al.* 1989). The release of catecholamines is not affected by propranolol (Fig. 4).

It is also possible that the chemoreceptors are stimulating breathing directly, and the release of catecholamines is simply coincidental. Milsom and Sadig (1983) demonstrated, in the rabbit, that propranolol caused a reduction of the chemoreceptor response to hypoxia, and a similar observation has been made in trout (M. L. Burleson, personal communication). This means that the correlations between V_G and circulating catecholamines observed in the present study could be spurious. If propranolol caused the peripheral chemoreceptors to decrease their response to hypoxia, there would be a lack of increase in V_G, and so the ventilatory responses observed would be similar to those recorded in the present study. Although this is a possibility, we think that it is unlikely for several reasons. First, at the environmental P_{O_2} we were working with, chemoreceptor activity is depressed, so it is unlikely that propranolol was blocking any stimulatory signals from the chemoreceptors. Second, propranolol blocked the ventilatory response to acid infusion. This could not have been due to stimulation of chemoreceptors, since Pa_{O_2} increased rather than decreased, so the activity of the chemoreceptors would be unchanged or possibly reduced. Finally, catecholamine infusion is known to stimulate ventilation during normoxia (Peyraud-Waitzenegger, 1979).

Control ventilation rates were different between treatments. Most of this difference is accounted for in the saline-treated fish, which had a significantly lower ventilation rate than the other treatments. This is probably due to the effect of temperature on trout; the average temperature in the hypoxia- only and propranolol-treated fish was 16±0.45°C, while the average temperature in the saline-treated trout was 12.7±0.17°C. Ventilation rates at elevated temperatures are higher than at lower temperatures (Hughes and Roberts, 1970).

The plasma acidosis observed in all fish during hypoxia was probably due to anaerobic respiration. Lactate levels were not measured, but other studies have indicated that, during deep hypoxia, there is an elevation of blood lactate concentration (Tetens and Lykkeboe, 1985; Boutilier *et al.* 1988), which is an indication of increased anaerobic metabolism (Holeton and Randall, 1967).

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