

EFFECTS OF CATECHOLAMINES ON GAS EXCHANGE AND VENTILATION IN RAINBOW TROUT (*SALMO GAIIRDNERI*)

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Accepted 19 April 1990

Summary

A transient inhibitory effect of catecholamines on relative CO_2 excretion, mediated by an inhibition of HCO_3^- dehydration through the red blood cell (RBC), has been proposed to cause the increase in Pa_{CO_2} routinely observed after strenuous exercise in fish (' CO_2 retention hypothesis', Wood and Perry, 1985). To evaluate this idea, trout fitted with arterial cannulae, oral membranes and opercular catheters were placed in ventilation chambers. Pa_{CO_2} , RBC intracellular pH (pHi) and other blood acid–base parameters were monitored from the arterial cannulae. The ventilation chamber system allowed continuous, almost instantaneous, measurements of water ΔO_2 and ΔCO_2 across the gills, and therefore of respiratory exchange ratio (RE), as well as $\Delta\text{ammonia}$, mean expired pH and ventilation volume (\dot{V}_w). Physiological doses of adrenaline and noradrenaline (3.2 nmol kg^{-1}), designed to duplicate typical post-exercise concentrations, together with appropriate saline controls, were injected into resting fish. Adrenaline caused an immediate hypoventilation, while the response to noradrenaline was biphasic: hyperventilation followed by hypoventilation. With both drugs, ΔO_2 and ΔCO_2 increased, but RE remained constant (adrenaline) or increased (noradrenaline). There was no evidence of a specific inhibition of CO_2 excretion, nor was there any increase in Pa_{CO_2} ; changes in RBC pHi were small (noradrenaline) or non-existent (adrenaline). These results confirm those of Steffensen *et al.* (1987) and do not support the CO_2 retention hypothesis. However, the RBCs of resting trout may be relatively insensitive to catecholamines at normal arterial blood pH (pHa).

Introduction

Fish routinely show large increases in Pa_{CO_2} after strenuous exercise, even though there is no corresponding decrease in Pa_{O_2} (Wood and Perry, 1985). The latter would be expected if the Pa_{CO_2} elevation were due to a simple diffusive or convective limitation on gas exchange. Wood and Perry (1985) and Perry (1986) proposed that the effect is due to an inhibition of HCO_3^- dehydration through the

Key words: catecholamines, CO_2 excretion, ventilation, red blood cell pHi, respiratory exchange ratio.

red blood cell (RBC), induced by the mobilization of catecholamines that accompanies severe exercise. A transient inhibition of relative \dot{M}_{CO_2} (i.e. a decrease in the respiratory exchange ratio, RE) would ensue until P_{aCO_2} had risen enough to restore the balance between CO_2 production and excretion rates. The effect would be analogous to a functional decrease in RBC carbonic anhydrase activity, and would result in a rise in P_{CO_2} levels throughout the animal, including the arterial blood, producing an apparent diffusive limitation on CO_2 excretion. Such increases in P_{aCO_2} have been observed in other treatments that reduce functional carbonic anhydrase activity, such as inhibition of the enzyme by acetazolamide (Haswell and Randall, 1978) or removal of the enzyme by experimental anaemia (Wood *et al.* 1982).

This CO_2 retention idea was supported by preliminary data of S. F. Perry and T. A. Heming (in Wood and Perry, 1985) showing that adrenaline inhibited the HCO_3^- -entry step into trout red cells *in vitro* by a β -adrenergic mechanism. Later, Perry and Vermette (1987) and Vermette and Perry (1988) reported that prolonged infusion of adrenaline into trout *in vivo*, to give typical post-exercise plasma concentrations (60 nmol l^{-1} ; see Milligan and Wood, 1987), resulted in a significant elevation of P_{aCO_2} . In contrast, Tufts *et al.* (1988) could find no effect of catecholamines on HCO_3^- flux through trout erythrocytes *in vitro*, and the rates were actually elevated in red cells taken from exhaustively exercised fish. Further doubt was cast on the CO_2 retention theory by Steffensen *et al.* (1987), who measured an increase, rather than a decrease, in RE after burst swimming in rainbow trout. In addition, Steffensen *et al.* (1987) found no effect of high doses of adrenaline ($50 \text{ nmol kg}^{-1} \approx 1000 \text{ nmol l}^{-1}$ in blood plasma) on directly measured \dot{M}_{CO_2} or RE. A possible limitation of these experiments was their temporal resolution. \dot{M}_{CO_2} was measured by sealing large-volume respirometers (38 l in the swimming studies, 3 l in the adrenaline injection studies) for 10–20 min. It is possible that a transient reduction of \dot{M}_{CO_2} or RE would have been undetectable in such large systems.

The principal goal of the present study was to re-examine the whole question of possible adrenergic effects on P_{aCO_2} , CO_2 excretion and RE by injecting physiological doses of catecholamines into otherwise resting trout while observing the responses with a measurement system of high temporal resolution. A further goal was to assess the effects of these physiological doses of adrenaline and noradrenaline on directly measured ventilation; there has been considerable controversy and speculation on this topic in recent years (reviewed by Perry and Wood, 1989).

The experimental approach was designed to avoid the effects of increased metabolic rate, metabolic acid production, the associated titration of HCO_3^- stores, the mobilization of endogenous catecholamines and other hormones and the occurrence of ram ventilation, all of which complicate the interpretation of responses in exercised fish (Wood and Perry, 1985). The dose of catecholamines ($3.2 \text{ nmol kg}^{-1} \approx 64 \text{ nmol l}^{-1}$ in blood plasma) was chosen to duplicate the typical plasma levels measured after exhaustive exercise in trout (Milligan and Wood,

1987). P_{aCO_2} , RBC pHi and other acid–base parameters in arterial blood were monitored from an indwelling catheter. The branchial siphon technique developed by Wright *et al.* (1986) and modified by Playle and Wood (1989) was used to monitor continuously the chemistry of expired water. This method yields nearly instantaneous measurements of mean expired CO₂, O₂, and therefore of RE, as well as mean expired pH, total ammonia and ventilation volume. Noradrenaline (in addition to adrenaline) was tested because it is reported to be more potent in eliciting β -adrenergic RBC intracellular pH (pHi) regulation (Tetens *et al.* 1988; Cossins and Kilbey, 1989). We suspected that an inhibition of HCO₃[−] flux into the RBC, and therefore a reduction in CO₂ excretion, could be directly or indirectly linked to β -adrenergic activation of Na⁺/H⁺ exchange on the RBC membrane.

Materials and methods

Preparation of experimental animals

Rainbow trout (*Salmo gairdneri* Richardson = *Oncorhynchus mykiss*; 200–350 g; $N=24$) were obtained from Spring Valley Trout Farm, New Dundee, Ontario, and held in dechlorinated Hamilton city tapwater. At least 14 days before experiments, the fish were transferred to flowing soft water which was made as described by Playle and Wood (1989). Temperature was $15\pm 1^\circ\text{C}$ and feeding was suspended during this acclimation period. A defined softwater medium (in $\mu\text{equiv l}^{-1}$: $\text{Ca}^{2+}\approx 50$, $\text{Na}^+\approx 50$, $\text{Cl}^-\approx 100$, titratable alkalinity ≈ 130 , pH 6.7) was used to provide a low total CO₂ background ($<100\ \mu\text{mol l}^{-1}$), against which CO₂ excretion by the fish could be measured. This medium was very similar in composition to the Vancouver tapwater used by Steffensen *et al.* (1987).

Fish were anaesthetized with MS-222 (Sigma, $0.4\ \text{mg l}^{-1}$, neutralized with KOH) and fitted with indwelling dorsal aortic cannulae (Soivio *et al.* 1972) to allow infusions and blood sampling with minimal disturbance. Latex ventilation masks (Davis and Cameron, 1971; Wright *et al.* 1986) were fitted to each fish to separate inspired and expired water. Opercular catheters were implanted to allow continuous monitoring of expired water chemistry, and the fish was then placed in a dark chamber for measurement of ventilation. Full details of the latter are given by Playle and Wood (1989). In brief, the opercular catheter was an 85 cm length of PE 190 tubing anchored about 1 cm anterior to the posterior margin of the operculum. The catheter was bent at 90° approximately 0.5 cm from the opercular surface. A flow of aerated water ($P_{O_2}>2\ \text{kPa}$, $P_{CO_2}<0.13\ \text{kPa}$) in excess of the fish's ventilatory demand was piped into the anterior (inspired) compartment of the ventilation chamber. Standpipes in the inspired and expired compartments of the chamber were set to identical heights so that the rear overflow provided a direct measure of the ventilation volume (\dot{V}_w). Fish were allowed to recover for 24–48 h before the start of experiments.

Experimental protocol

There were three injection series ($N=8$ for each): control ($1.0\ \text{ml kg}^{-1}$ of

140 mmol l⁻¹ NaCl); adrenaline (3.2 nmol kg⁻¹ of Sigma 1-epinephrine bitartrate in 0.5 ml kg⁻¹ of 140 mmol l⁻¹ NaCl); and noradrenaline (3.2 nmol kg⁻¹ of Sigma 1-arterenol bitartrate in 0.5 ml kg⁻¹ of 140 mmol l⁻¹ NaCl). An additional wash-in of 0.5 ml kg⁻¹ of 140 mmol l⁻¹ NaCl was used for the catecholamine injections, so the total infusion volume was the same for all three series. Fresh adrenaline and noradrenaline solutions were made for each experiment. Arterial blood samples (480 µl) were drawn before (initial) and at 4 and 14 min after the injections. A period of 20–25 min intervened between the initial sample and the injection (time 0). In addition to providing pre-infusion blood data, the initial sample served to assess the influence of blood sampling alone on the measured ventilatory and expired water chemistry parameters. Blood samples were analyzed for whole-blood pHa, plasma total CO₂ concentration, PaO₂ and RBC pHi.

Ventilation volume (\dot{V}_w) and mean expired water parameters were measured continuously for 15 min after the initial blood sample, and continuously for 25 min after injections. Water was analyzed for pH, CO₂, O₂ and total ammonia content. Measurements of inspired water parameters, taken from an identical catheter placed in front of the fish's mouth, were taken periodically to ensure constant inhalant water conditions.

Analytical techniques

Blood samples were analyzed for pHa (Radiometer E5021) and PaO₂ (Radiometer E5046) using standard electrode techniques and a Radiometer pHM 72 acid–base analyser. True plasma was obtained by centrifuging blood at 10 000 g for 2 min, and analysed for total CO₂ with a Corning 965 CO₂ analyser. RBC pHi was measured on the red cell pellet by the freeze–thaw method of Zeidler and Kim (1977). PaCO₂ was calculated using the Henderson–Hasselbalch equation and values of pK' and αCO₂ for rainbow trout plasma from Boutilier *et al.* (1984). Ventilation volume (\dot{V}_w) was measured directly by collecting the overflow of the rear compartment over 1 min intervals.

The siphon rate of the inspired and opercular catheters was approximately 3.0 ml min⁻¹. Flow was directed first to a small polyethylene vial into which were sealed the bulb of a Radiometer GK2401C pH electrode (connected to a pHM 72 acid–base analyser) and a magnetic flea for continuous stirring. From there the water passed to a Radiometer D616 thermostatted cell, housing an E5046 P_{O₂} electrode (connected to a Cameron Instruments OM-200 P_{O₂} meter), and finally to a 2 ml screw-top glass vial for sequential 1 min collection of samples for total CO₂ and ammonia analyses. The water flow was discharged at the bottom of this vial and the excess volume allowed to overflow at the top, so that air was excluded. The vial was tightly capped and the total CO₂ content was determined on a 1 ml subsample within 4 h. The remainder was frozen at –20°C for later determination of total ammonia content. Total volume of the siphon and electrode system (excluding the glass vial) was 2.6 ml. Response delays of the system were measured by the application of step changes in P_{O₂}, CO₂ and pH, and were taken into account in analysis of the data. Temporal resolution was approximately 1 min,

which corresponded to the collection period for the CO₂, ammonia and $\dot{V}w$ analyses.

The output of the pH and P_{O_2} electrodes was continuously displayed on a dual-channel chart recorder (Brown Boveri and Co. SE120). Calibration was done *in situ* under flowing conditions. Tests in which the ionic strength of the softwater medium was adjusted to that of the calibration buffers indicated that pH was underestimated by about 0.05 unit. Water P_{O_2} values (kPa) were converted to O₂ concentrations ($\mu\text{mol l}^{-1}$) using the solubility of O₂ at 0% salinity (Boutilier *et al.* 1984). Total CO₂ analyses were made by shaking 1.0 ml of water with 0.5 ml of helium-equilibrated HCl (0.1 mol l^{-1}) and 4.5 ml of helium in a Hamilton gas-tight syringe. The extracted gas was injected into a Shimadzu GC-8A gas chromatograph equipped with a Poropak Q column, and the output displayed on a Shimadzu-CR3A integrator. Unknowns were determined against identically extracted standards of 0, 100, 200 and $300 \mu\text{mol l}^{-1}$ NaHCO₃. Total ammonia was assayed by the salicylate–hypochlorite method (Verdouw *et al.* 1978).

Water O₂, CO₂ and ammonia data have been expressed as instantaneous values or 'transfer', i.e. the difference between inspired and expired concentrations. The instantaneous gas exchange ratio at the gills (RE) was calculated as $\Delta\text{CO}_2/\Delta\text{O}_2$. Data have been routinely expressed as means \pm 1 s.e.m. (*N*). Statistical comparisons have been made using Student's two-tailed paired *t*-tests at $P \leq 0.05$, using each fish as its own control.

Results

Ventilation volume ($\dot{V}w$) was unaffected by blood sampling or the injection of NaCl alone (Fig. 1A). Very minor changes in ventilatory pattern accompanying blood sampling, which can sometimes be seen on pressure or impedance records, would be damped by the mask used for $\dot{V}w$ measurement. Adrenaline (3.2 nmol kg^{-1}) caused an immediate hypoventilation of about 10%, which was significant only at 0–1 min but persisted until 4 min in most fish; thereafter, $\dot{V}w$ returned to pre-injection levels (Fig. 1B). In contrast, noradrenaline (3.2 nmol kg^{-1}) caused an immediate hyperventilation of about 15%, which also persisted until 4 min in most fish. This was followed by a 10% hypoventilation, significant at 8–10 min, with a variable rate of recovery thereafter.

Branchial O₂ transfer tended to fall slightly during the first few minutes after blood sampling in all groups, but this response was significant only in the control group (Fig. 2A). The injection of NaCl itself had no significant effect. Both adrenaline and noradrenaline significantly increased ΔO_2 by 15–20% in the first 5 min post-injection (Fig. 2B,C). This could reflect a simple flow-dependent effect when $\dot{V}w$ was decreased by adrenaline. However, flow-dependency cannot be the explanation for the increase in ΔO_2 caused by noradrenaline, which increased rather than decreased $\dot{V}w$ at this time.

Branchial CO₂ transfer was not altered by blood sampling, although it did fall slightly immediately after the injection of NaCl (Fig. 3A). As with ΔO_2 , ΔCO_2

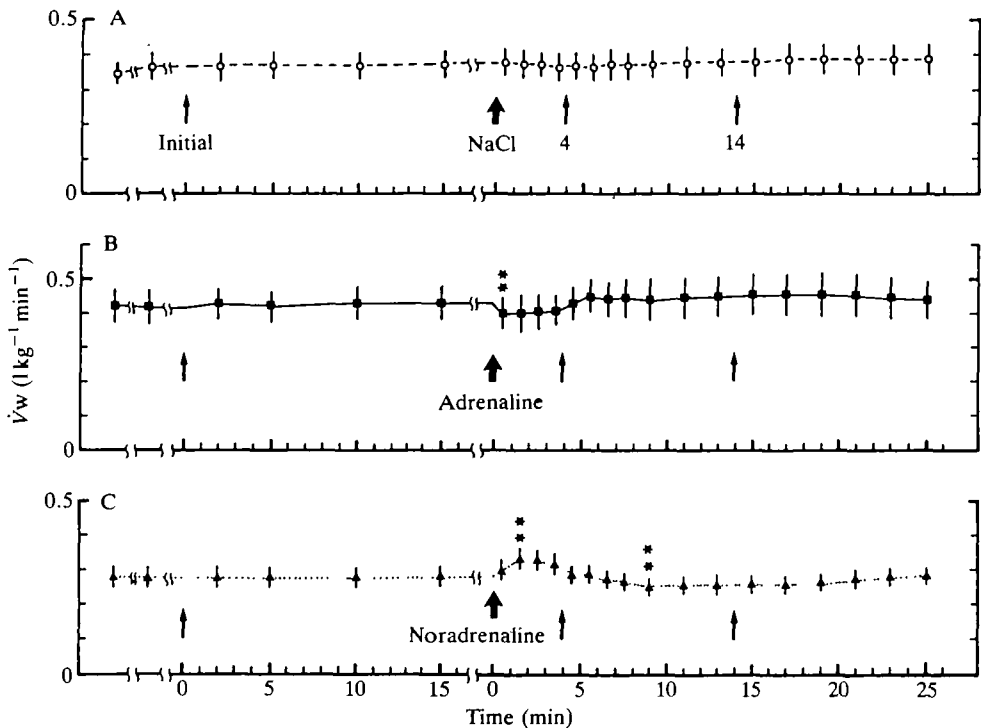


Fig. 1. The influence of arterial injection of saline vehicle alone (1.0 ml kg^{-1} of 140 mmol l^{-1} NaCl), adrenaline (3.2 nmol kg^{-1} in 1.0 ml kg^{-1} of 140 mmol l^{-1} NaCl) and noradrenaline (3.2 nmol kg^{-1} in 1.0 ml kg^{-1} of 140 mmol l^{-1} NaCl) on ventilation volume (\dot{V}_w) in rainbow trout. Large arrows represent the time of injection, and small arrows the time of blood sampling before (initial) and at 4 and 14 min after the injection. Tests of significance compare values after the initial blood sample with the mean value before it, and values after injection with the mean of pre-injection but post-initial blood sampling values. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Means ± 1 S.E.M. ($N=7-8$ for each group).

was elevated significantly by both adrenaline and noradrenaline during the first 5 min post-injection (Fig. 3B, C). However, in most fish the stimulation of CO_2 transfer lasted longer than the stimulation of O_2 transfer. This effect was especially pronounced for noradrenaline (Fig. 3C).

The instantaneous RE value calculated from these ΔCO_2 and ΔO_2 data was initially about 0.9 in all three groups. RE was unaffected by blood sampling, but fell slightly after NaCl administration (Fig. 4A), reflecting the transient decrease in ΔCO_2 (Fig. 3A). Despite this fall in RE due to the NaCl vehicle alone, neither catecholamine caused any decrease in RE. On the contrary, RE increased significantly to a value of 1.0–1.2 over the period 4–8 min after the administration of noradrenaline (Fig. 4C). This was both preceded and followed by non-significant increases for several more minutes in most fish. There was also a slight tendency for RE to rise after adrenaline infusion, with a significant increase

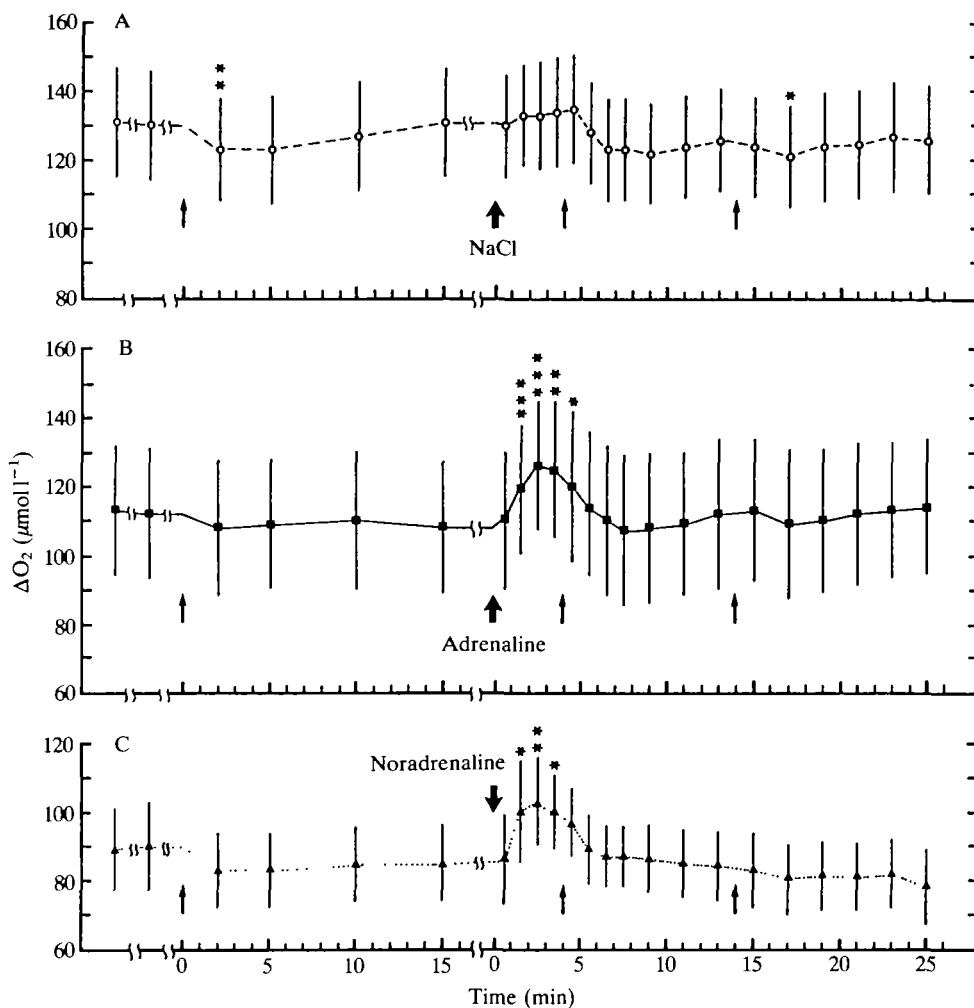


Fig. 2. The influence of saline vehicle, adrenaline and noradrenaline on the transfer of oxygen (ΔO_2 =difference in O_2 concentration between inspired and expired water) across the gills of rainbow trout. Other details as in legend of Fig. 1.

8–10 min post-injection (Fig. 4B). There was no evidence of a relative inhibition of CO_2 excretion (i.e. decrease in RE) caused by these physiological doses of catecholamines.

There were no significant changes in expired water pH in response to blood sampling or any of the infusion treatments, and no differences between the treatment groups, so the data have not been illustrated. Mean expired pH stayed constant at 6.2–6.3, approximately 0.4 units below inspired pH. Branchial ammonia transfer (data not shown) was $15\text{--}30 \mu\text{mol l}^{-1}$ initially and was unaffected by blood sampling; this value increased by 25–50 % at 2–5 min in all groups, apparently a non-specific response to the injection of NaCl.

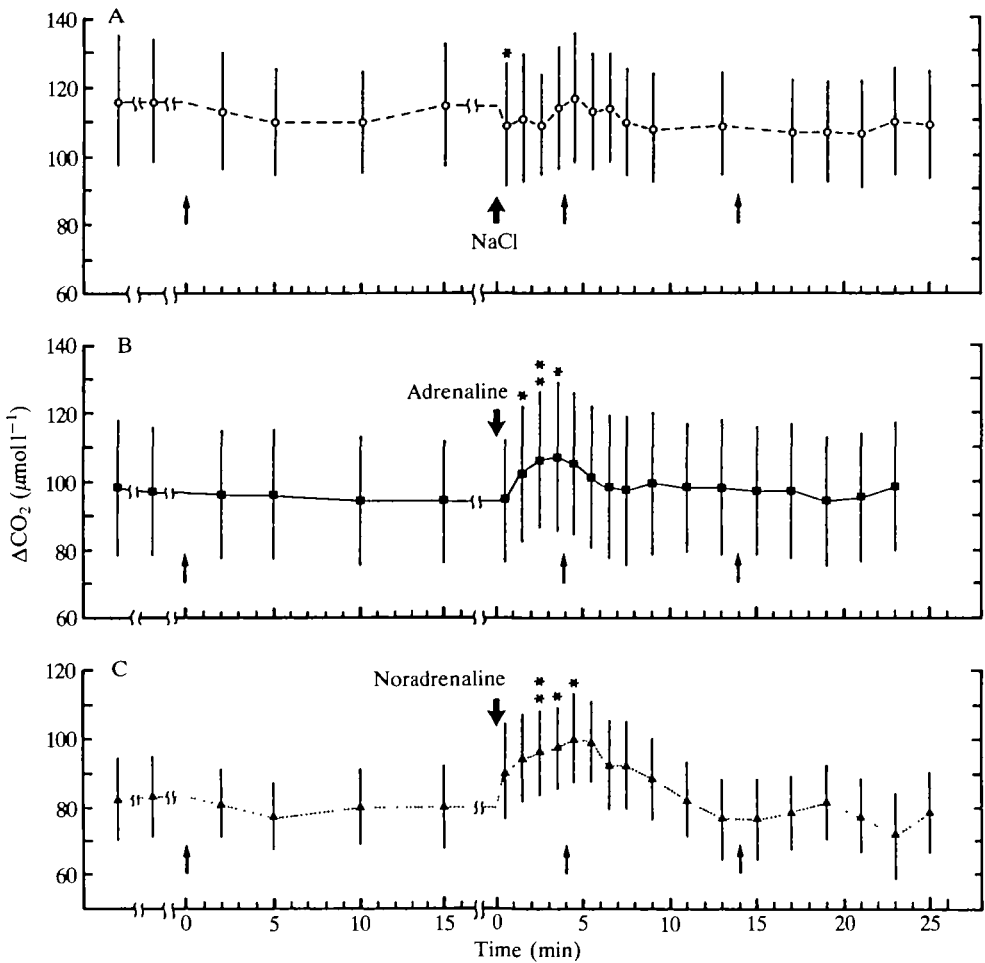


Fig. 3. The influence of saline vehicle, adrenaline and noradrenaline on the transfer of carbon dioxide (ΔCO_2 =difference in total CO_2 concentration between expired and inspired water) across the gills of rainbow trout. Other details as in legend of Fig. 1.

Blood gas data provided no evidence that either adrenaline or noradrenaline caused a retention of CO_2 in the blood. P_{aCO_2} was unchanged at 4 min and fell slightly at 14 min after administration of catecholamines, especially noradrenaline (Fig. 5A). As this effect was also seen in the control group, it was at least partially a non-specific response to blood sampling or NaCl infusion. P_{aO_2} remained unchanged in the control and noradrenaline groups, but increased significantly 14 min after the injection of adrenaline (Fig. 5B). Plasma total CO_2 content stayed constant in all three groups (Fig. 5C).

Arterial blood pH increased significantly 4 and 14 min after noradrenaline infusion (Fig. 5D), in accordance with the calculated reduction of P_{aCO_2} (Fig. 5A); similar trends were seen in the control and adrenaline-treated groups

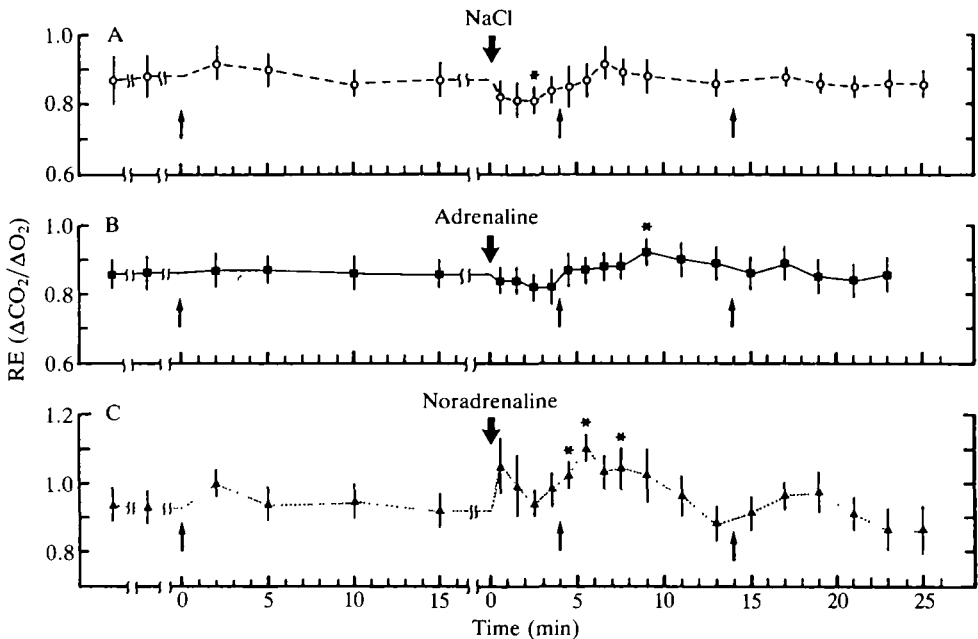


Fig. 4. The influence of saline vehicle, adrenaline and noradrenaline on the instantaneous respiratory gas exchange ratio ($\text{RE} = \Delta\text{CO}_2/\Delta\text{O}_2$) across the gills of the rainbow trout. Other details as in legend of Fig. 1.

(Fig. 5D). RBC pHi was slightly but significantly elevated 4 min after noradrenaline infusion, but there were no other significant changes in pHi (Fig. 5E). There were also no significant changes in the extracellular to intracellular pH gradient ($\text{pHa} - \text{pHi}$) across the RBC membrane, which stayed constant at about 0.5 units during all three treatments (Fig. 5F).

Discussion

Chemistry of expired water: comparison with previous studies

Although there have been numerous studies of $\dot{V}\text{w}$ and O_2 extraction in trout gills, only a few have also included measurements of CO_2 , ammonia and pH in the expired water (Wright *et al.* 1986; Iwama *et al.* 1987; Playle and Wood, 1989). In general, the present mean values for resting trout at 15°C in soft water ($\Delta\text{O}_2 = 108 \mu\text{mol l}^{-1}$, $\Delta\text{CO}_2 = 96 \mu\text{mol l}^{-1}$, $\Delta\text{ammonia} = 20 \mu\text{mol l}^{-1}$, expired $\text{pH} = 6.2$, inspired $\text{pH} = 6.6$, $\dot{V}\text{w} = 0.36 \text{ l kg}^{-1} \text{ min}^{-1}$) agree well with values obtained under comparable conditions in the three previous studies. At inspired $\text{pH} = 6.6$, the decrease in pH as water passes over the gills is the net result of an acidification by CO_2 and a lesser alkalization by ammonia and titratable base; the expired pH is at or close to equilibrium. The phenomenon has been modelled by Playle and Wood (1989). The present mean RE value (0.88) at the gills is lower

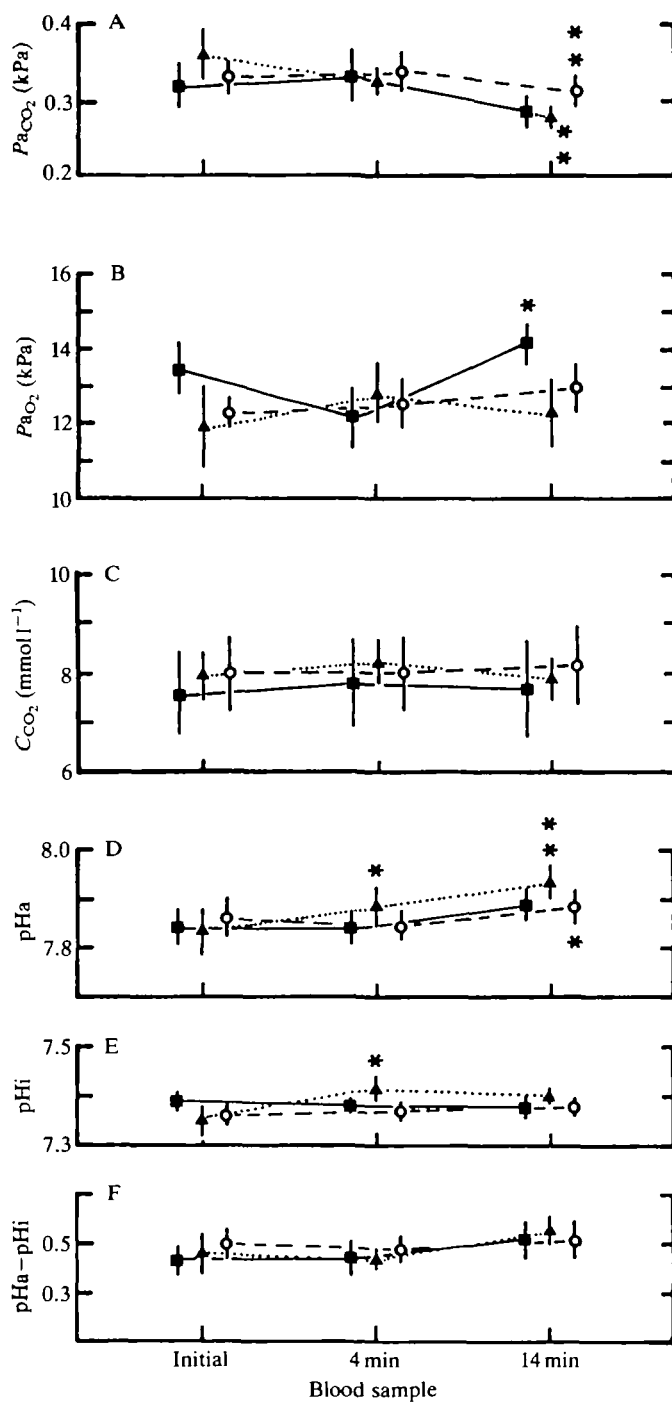


Fig. 5

Fig. 5. The influence of saline vehicle (○), adrenaline (■) and noradrenaline (▲) on blood gases and acid–base status in rainbow trout. (A) Arterial carbon dioxide tension (Pa_{CO_2}); (B) arterial oxygen tension (Pa_{O_2}); (C) plasma total carbon dioxide concentration (C_{CO_2}); (D) arterial pH (pH_a); (E) red blood cell intracellular pH (pH_i); and (F) the pH gradient across the RBC membrane ($\text{pH}_\text{a}-\text{pH}_\text{i}$). Other details as in legend of Fig. 1.

than that reported by Playle and Wood (1989; $\text{RE}=1.18$) using very similar methods, but identical to that measured by Iwama *et al.* (1987; $\text{RE}=0.87$), who sampled expired water from a mixing tube around the fish. It is possible that the trout of Playle and Wood (1989) were not in steady-state conditions. There have been no previous measurements of the effects of catecholamines on RE , ΔO_2 , ΔCO_2 , $\Delta\text{ammonia}$ or mean expired pH in trout gills.

Ventilatory responses to catecholamine infusions

The role of catecholamines in the normal control of ventilation in fish has been a topic of considerable speculation in recent years (reviewed by Perry and Wood, 1989). In the present study, relatively low doses of adrenaline and noradrenaline had rapid effects on \dot{V}_w when injected into the bloodstream, illustrating their potential for a physiological role. Adrenaline caused hypoventilation, while the response to the same dose of noradrenaline was biphasic – hyperventilation followed by hypoventilation (Fig. 1).

In the eel, Peyraud-Waitzenegger *et al.* (1980) found that similar doses of catecholamines caused β -adrenergic hyperventilation and α -adrenergic hypoventilation, with the former predominating in summer and the latter in winter. Noradrenaline was more potent in inducing the β -adrenergic hyperventilation (suggesting a β_1 response), and adrenaline was more potent in inducing the α -adrenergic hypoventilation. In the present study, the possible role of seasonality is unclear, because the noradrenaline experiments were run in winter (December) and the adrenaline experiments in both summer (June–July) and winter (December) with similar results. R. Kinkead and S. F. Perry (personal communication) have recently found that doses of both catecholamines slightly higher than those used by us caused only hypoventilation in trout in summer. The simplest conclusion is that the dominant response in trout is hypoventilation, though noradrenaline may cause an additional rapid hyperventilation. Nekvasil and Olson (1986) concluded that, for trout, there was a blood–brain barrier for adrenaline but not for noradrenaline, so differential effects on central adrenoreceptors are one possible explanation for their distinct actions on ventilation.

The effect of catecholamines on RBC pH_i and Pa_{CO_2}

The injection of physiological doses of catecholamines caused either no response or only a small response in RBC pH_i (Fig. 5E). About half of the slight but significant increase in pH_i induced by noradrenaline was associated with the simultaneous rise in extracellular pH (pH_a) and decrease in Pa_{CO_2} (Fig. 5A,D).

However, a quantitative consideration of the relative buffering capacities of RBCs *versus* plasma, based on data in Wood *et al.* (1982), Heming *et al.* (1986) and Motais *et al.* (1989), suggests that this cannot be the complete explanation, and that a small rise in pHi of metabolic origin must have been responsible for the other half (0.03–0.04 pH units). Inasmuch as the same dose of adrenaline was without influence on RBC pHi, the results support the conclusion of Tetens *et al.* (1988) that noradrenaline is a more potent activator of RBC Na^+/H^+ exchange.

However, these effects on RBC pHi were much smaller than reported in a number of previous studies both *in vivo* and *in vitro* (Steffensen *et al.* 1987; Perry and Vermette, 1987; Cossins and Kilbey, 1989; McDonald *et al.* 1989; additional examples cited in Wood and Perry, 1985, and Perry and Wood, 1989). This difference can be explained by the fact that the present study used low, physiological doses in resting, normoxic fish. Other studies used far higher or more prolonged doses, or the highly potent synthetic catecholamine isoprenaline, or administered the catecholamines under conditions where the fish was stressed and the blood was already acidotic or hypoxaemic. Recently, it has become clear from *in vitro* studies that the Na^+/H^+ exchanger responsible for the RBC pHi response is relatively insensitive to adrenergic stimulation at normal plasma pH, but becomes markedly sensitized at acidic pH values representative of typical post-exercise conditions, as first shown by Nikinmaa *et al.* (1987) and confirmed by others (Borgese *et al.* 1987; Cossins and Kilbey, 1989; Milligan *et al.* 1989). Hypoxaemia has a similar potentiating effect (Motais *et al.* 1987).

In contrast to the predictions of the CO_2 retention theory, the administration of physiological doses of catecholamines to resting trout did not cause any increase in PaCO_2 (Fig. 5A). Indeed, slight decreases were observed in both control and experimental groups. The difference between these results and those of Perry and Vermette (1987) and Vermette and Perry (1988), who found an increase in PaCO_2 , may be related to the prolonged infusion of adrenaline used by these workers. Notably, their fish also exhibited extracellular acidosis and marked increases in RBC pHi. If elevation of PaCO_2 is in any way linked to the β -adrenergic pHi response, it would have been favoured by the conditions of their experiments.

Effect of catecholamines on CO_2 excretion

The present methods would undoubtedly have detected even a transient decrease in RE at the gills, had it occurred. There was no decrease in RE induced by catecholamines. On the contrary, RE tended to increase, especially after noradrenaline infusion (Fig. 4). These results therefore support the general conclusions of Tufts *et al.* (1988), which were based on a very different experimental approach, the measurement of HCO_3^- flux through trout erythrocytes. More directly, they also support the findings of Steffensen *et al.* (1987). The administration of physiological doses of catecholamines to resting trout does not cause CO_2 retention, and the absence of such a phenomenon in the study of Steffensen *et al.* (1987) was not due to the use of large-volume systems or much higher doses of adrenaline. Our data provide no support for the idea that observed post-

exercise increases in P_{aCO_2} are caused by a catecholamine-induced inhibition of HCO_3^- dehydration through the RBC, and therefore of relative CO₂ excretion, as proposed by Wood and Perry (1985) and Perry (1986).

The observed increase in ΔCO_2 (Fig. 3) and RE (Fig. 4) after noradrenaline injection was correlated with the decrease in P_{aCO_2} (Fig. 5A). The phenomenon was not due to metabolic acidosis and a resulting titration of plasma HCO_3^- , the usual explanation for eventual increases in RE after strenuous exercise (Wood and Perry, 1985; Steffensen *et al.* 1987). Indeed, pH_a increased (Fig. 5D). Instead, increased RE and decreased P_{aCO_2} may have resulted from the increase in branchial gas transfer capacity which catecholamines cause (Randall and Daxboeck, 1984), coupled with the greater diffusibility of CO₂ than O₂. In this regard, it is notable that noradrenaline, which caused the greatest rise in RE (Fig. 4), is more potent than adrenaline in β -adrenergic vasodilation of the gills in trout (Wood, 1975), and that systemic β -blockade with propranolol greatly increases P_{aCO_2} (Vermette and Perry, 1988; McDonald *et al.* 1989).

While the present results do not support the CO₂ retention hypothesis, they do not disprove it conclusively, for the following reasons. The present study employed low *physiological* doses of adrenaline and noradrenaline under *resting*, *non-acidotic* conditions. The adrenergic retention of CO₂ *in vivo*, which would cause decreased RE and increased P_{aCO_2} , is proposed to occur under the acidotic conditions that exist in the blood after strenuous exercise (Wood and Perry, 1985; Perry, 1986). The RBC pH_i response would be potentiated by extracellular acidosis (Nikinmaa *et al.* 1987; Borgese *et al.* 1987; Cossins and Kilbey, 1989; Milligan *et al.* 1989); indeed, *in vivo* measurements demonstrate that Na⁺/H⁺ exchange on the RBC membrane is strongly activated at this time by physiological concentrations of endogenously mobilized catecholamines (Wood and Perry, 1985; Milligan and Wood, 1987). The small size or absence of this response in the present experiments may indicate that resting conditions are not appropriate for studying phenomena which normally occur after exercise. This would be particularly true if the P_{aCO_2} rise were linked directly or indirectly to the RBC pH_i response. Unfortunately, it would be very difficult to mimic post-exercise conditions in fish confined in ventilation boxes, and even more difficult to do so without eliciting the mobilization of endogenous catecholamines.

This research was supported by an NSERC operating grant to CMW. RCP held an NSERC Postgraduate Scholarship.

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