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THE ROLE OF β-ADRENORECEPTORS IN THE RECOVERY FROM EXHAUSTIVE EXERCISE OF FRESHWATER-ADAPTED RAINBOW TROUT

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Summary

Rainbow trout, fitted with arterial catheters, were exercised to exhaustion by manual chasing and then injected with either saline (controls), the β-agonist isoproterenol or the β-antagonist propranolol. Blood acid–base status, branchial unidirectional and net fluxes of Na^+ and Cl^-, and net fluxes of ammonia and acidic equivalents (J^H_{net}) were monitored over the subsequent 4 h of recovery. These same parameters were also monitored in normoxic, resting fish following isoproterenol injection and in exercised fish following acute post-exercise elevation of external NaCl concentration. In addition to confirming an important role for β-adrenoreceptors in the regulation of branchial gas exchange and red cell oxygenation and acid–base status, we find a significant β-adrenergic involvement in the flux of lactic acid from muscle and in J^H_{net} across the gills. Both isoproterenol infusion (into non-exercised fish) and exhaustive exercise were found to cause net acid excretion. The post-exercise J^H_{net} was further augmented by elevating [NaCl] but was not affected, in this instance, either by β-stimulation or blockade, indicating that J^H_{net} was not entirely regulated by a β-adrenergic mechanism. On the basis of a detailed analysis of unidirectional Na^+ and Cl^- fluxes, we conclude that the increase in J^H_{net} following exercise arose mainly from increased $Na^+/H^+(NH_4^+)$ exchange and that the upper limit on J^H_{net} was set by the supply of external counterions and by the increase in branchial ionic permeability that invariably accompanies exhaustive exercise.

Introduction

Several studies have demonstrated that exhaustive exercise has a profound physiological impact on the rainbow trout (see Wood and Perry, 1985, for a review). A severe blood acidosis of mixed metabolic and respiratory origin is produced, and corrected in part, by excretion of acidic equivalents by the gills (Holeton *et al.* 1983; Milligan and Wood, 1986*a*; Wood, 1988). Associated with this

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disturbance is a considerable elevation in levels of plasma catecholamines (particularly adrenaline), which occurs in proportion to the magnitude of the disturbance (Tang and Boutilier, 1988). This catecholamine surge is thought to aid in the restoration of acid–base and respiratory homeostasis, largely through the activation of β-adrenergic receptors.

β-receptors are implicated in at least four processes relevant to the defence against and recovery from stress: regulation of branchial gas transfer (Peyraud-Waitzenegger, 1979; Tetens and Christensen, 1987); the maintenance of red cell pH and therefore blood O_2 transport (Boutilier *et al.* 1986; Primmett *et al.* 1986; Milligan and Wood, 1987); carbohydrate metabolism, including the processing of lactic acid (Wardle, 1978; Mazeaud and Mazeaud, 1981); and the transfer of ionic and acid–base equivalents at the gills (Girard and Payan, 1980; Vermette and Perry, 1987). There is good evidence for the first two processes but the evidence for the latter two is indirect and/or contradictory. Consequently, the main objective of the present study was a detailed investigation of recovery of the trout from exhaustive exercise with the particular aim of clarifying the role, if any, that catecholamines play in lactic acid kinetics and branchial net acid transfers. To discriminate specific β-adrenoreceptor effects from others occurring in exercise recovery, blood and branchial responses were compared amongst animals injected post-exercise with either saline, isoproterenol (specific β-agonist) or propranolol (β-antagonist). β-adrenergic effects were also studied by injecting non-exercised fish with isoproterenol. A secondary objective of this study was to explore some of the other factors controlling or limiting net acid transfer at the gills. To this end, the effect of external ions on net acid transfer was studied by acutely elevating the ambient [NaCl] post-exercise.

Materials and methods

Experimental animals

Freshwater-adapted rainbow trout (155–349 g) were obtained from Merlin Fish Farms, Wentworth, Nova Scotia, and were maintained with daily feeding for at least 2 weeks in dechlorinated Halifax city tap water ($[Na^+]$=0.3; $[Cl^-]$=0.2; $[HCO_3^-]$=0.5 mequiv l^{-1}; pH=7.5–7.7; 6–9°C). Prior to use, the animals were transferred to a 560-l Living Stream tank (Frigid Unit Inc., USA) where they were acclimated to the experimental temperature (10±0.2°C) for about 1 week. Feeding was suspended 4 days prior to surgery. Under MS-222 anaesthesia (1:10000 in $NaHCO_3$-buffered fresh water), fish were fitted with dorsal aortic catheters. Following surgery, fish were placed into 2.8-l darkened Lucite flux boxes supplied with flowing aerated water thermostatted to 10±0.2°C. The flux boxes are designed so that vigorous aeration of an outer chamber provides convective mixing of water throughout an inner animal chamber; an aeration rate of 1 l min^{-1} ensured complete mixing of chamber contents within 1 min (see McDonald and Rogano, 1986, for details). Experiments were begun after the animals had had at least 48 h to recover from the surgery.

Experimental protocol

Arterial blood chemistry (series A–D, below) and transbranchial ion and 'acid' fluxes (series A, B and D only) were measured prior to and following exercise or other treatments. Flux measurements were begun by closing the inflow and outflow of the flux boxes and adjusting box volume to a standardized value (approximately 12 l kg^{-1} fish). The box was maintained at the experimental temperature (10±0.2°C) by being bathed in thermostatted water. Radiotracers (^{24}Na, 150 kBq; ^{36}Cl, 75 kBq) were added to the water and allowed to mix for 10 min before the first water sample was withdrawn. Flux measurements were made for 0.5–2 h intervals.

The following four experimental series were performed.

Series A. Isoproterenol infusion – no exercise

Trout (N=10) were infused with 2 μmol kg^{-1} of isoproterenol. Ion and 'acid' flux measurements were made for 1 h prior to and 4 h following infusion. Arterial blood samples (drawn 30 min prior to infusion, and at 10 min, 40 min, 70 min, 2 h and 4 h post-infusion) were analysed for pH (pHa), total CO_2, haematocrit and lactate.

Series B. Exercise with drug infusion and ion/H^+ fluxes

Trout (N=23) were exercised to exhaustion by 10 min of manual chasing in a cylindrical container. Immediately following exercise, fish were returned to flux chambers and injected with isoproterenol (2 μmol kg^{-1}, N=10), propranolol (1.7 μmol kg^{-1}, N=6) or saline (N=7, controls). Arterial blood samples and water samples were collected and analysed as in series A.

Series C. Exercise with drug infusion, no fluxes

Trout were exercised and infused as in series B (N=5 for each treatment). Arterial blood samples (drawn 30 min prior to exercise and at 10 min, 1 h, 2 h and 4 h post-exercise) were analysed for pHa, red cell pH, total CO_2, total O_2, Pa_{O_2} and haemoglobin concentration.

Series D. Exercise with post-exercise elevation in ambient NaCl

Trout (N=9) were exercised and sampled as in series B. Immediately following exercise, NaCl was added to the water to increase external [NaCl] from 0.3 to 1.3 mmol l^{-1}.

Analytical procedures

Blood

Arterial pH was determined using a microcapillary pH electrode (Radiometer G279/G2) coupled with a Radiometer PHM 72 acid–base analyser. The total CO_2 of anaerobically obtained whole blood (in mmol l^{-1}) was measured on 50 μl samples using a Corning (model 965) CO_2 analyser. Whole-blood lactate concen-

trations (in mequiv l^{-1}) were analysed by the L-lactate dehydrogenase/NADH method using Sigma reagents. Measurements of whole-blood total CO_2 were used to calculate arterial CO_2 tension (Pa_{CO_2}) and whole-blood [HCO_3^-] using experimentally determined pK′ and CO_2 solubility coefficients for trout plasma (Boutilier *et al.* 1984).

The concentration of metabolic protons added to whole blood ($\Delta[H^+]_m$) over any time period (1 to 2) was calculated from pHa and whole-blood [HCO_3^-] from the equation of McDonald *et al.* (1980*a*):

$$\Delta[H^+]_m = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2)\,, \quad (1)$$

where β, the non-bicarbonate buffer value of whole blood, is estimated from the haematocrit value at time 1 using the relationship determined by McDonald *et al.* (1980*b*):

$$\beta = -0.246 \times \text{haematocrit} - 3.97\,. \quad (2)$$

Oxygen tension of arterial blood (Pa_{O_2} in kPa) was determined with a Radiometer E5046 P_{O_2} electrode connected to a Radiometer PHM 72 meter. Total O_2 (mmol l^{-1}) was determined by the Tucker method (Tucker, 1967). Haemoglobin concentration (g dl^{-1}) was determined by Drabkin's method using Sigma reagents. Red cell pH was determined by the freeze–thaw method of Zeidler and Kim (1977).

Water

Water [Na^+] was measured with a Corning model 410 flame photometer. Water [Cl^-] was determined colorimetrically using the mercuric thiocyanate method (Zall *et al.* 1956). Water radioactivity was determined on 5 ml water samples added to 10 ml of Aqueous Counting Scintillant (ACS, Amersham Inc.) and counted in an LKB scintillation counter. Samples were first counted for total β activity ($^{24}Na + {}^{36}Cl$) and then re-counted 3–4 weeks later (after ^{24}Na had decayed) for ^{36}Cl activity. Water [ammonia] was measured by a micromodification of the salicylate–hypochlorite assay (Verdouw *et al.* 1978). Titratable alkalinity (TA) of water (used to estimate J_{net}^{H}, see below) was determined by titrating 5 ml water samples to pH 4.0 with 0.02 mol l^{-1} HCl according to the method of McDonald and Wood (1981). Net fluxes (J_{net}) of Na^+, Cl^-, ammonia and TA were calculated from their respective changes in concentration in water with time. Unidirectional fluxes of Na^+ and Cl^- (J_{in} and J_{out} in μequiv kg^{-1} h^{-1}) were calculated as follows:

$$J_{in} = \frac{Q^*i - Q^*f}{SA \cdot m \cdot t}\,, \quad (3)$$

$$J_{out} = J_{net} - J_{in}\,. \quad (4)$$

Where Q* is the total radioactivity in the water (in cts min^{-1}) at the beginning (i) and end (f) of the flux period, SA is the specific activity in the medium (in cts min^{-1} μequiv^{-1}), m is the animal's body mass in kilograms and t is the duration of the flux period in hours.

Net acid flux (J^{H}_{net}) was determined as the difference between the net ammonia flux and the net TA flux (signs considered).

Data have been expressed as means (±1 S.E.M.) throughout. Statistical comparisons within treatments were made with Student's paired *t*-test, using each animal as its own control with a significance level of $P<0.05$. Statistical comparisons between treatments were made with the unpaired *t*-test.

Results

Blood acid–base status

Effects of isoproterenol infusion (series A)

Bolus infusion of isoproterenol (2 μmol kg^{-1}) had, by itself, a pronounced and persistent effect on blood acid–base status (Fig. 1). Within 30 min of infusion, arterial pH (pHa) rose significantly above pre-infusion levels and remained elevated for almost 4 h (Fig. 1A). This alkalosis came about for two reasons: a significant reduction in Pa_{CO_2} levels (Fig. 1C) and a net reduction in the total fixed acids buffered by blood ($\Delta[H^+]_m$, Fig. 1E). The latter occurred despite a gradual rise in blood [lactate] (Fig. 1D).

Effects of exercise (series B, C and D)

Following exhaustive exercise, all fish, irrespective of treatment, exhibited similar blood acid–base disturbances: an acute reduction in arterial pH (Figs 2A, 3A, 4A) recovering to near normal by 4 h and jointly caused by a short-term elevation in arterial P_{CO_2} (Figs 2B, 3C, 4B) and a more prolonged release of lactic acid to the blood (Figs 2C,D, 4C,D). Typically, a discrepancy progressively developed between lactate and buffered fixed H^+ ($\Delta[H^+]_m$, Fig. 2D) such that by 4 h lactate exceeded H^+ by about 5 mequiv l^{-1} (Fig. 2E). All these features of the acid–base disturbances of exercise are quite similar to those reported previously for rainbow trout (Milligan and Wood, 1986*a*; Wood, 1988; Tang *et al.* 1989). Red cell pH was also significantly depressed following exercise (0.14 units compared to 0.38 units for pHa, Fig. 3B), recovering with the same time course as pHa. Along with the reduction in red cell pH was a significant reduction in haemoglobin O_2 content relative to pre-exercise levels (Fig. 3E); a depression that was partially offset by a significant elevation in Pa_{O_2} above pre-exercise levels (Fig. 3D).

On top of these major exercise-induced changes in arterial gases and acid–base status, there were significant effects of both β-adrenoreceptor stimulation (by isoproterenol, 2 μmol kg^{-1}) and β-receptor blockade (by propranolol, 1.7 μmol kg^{-1}) relative to untreated controls (Figs 2, 3). Both experiments with adrenergic effectors (series B and C) showed the same basic pattern of responses to either β-stimulation or blockade but there were some slight statistical differences in adrenergic effects between the two series. Nonetheless, isoproterenol infusion typically affected both arterial P_{CO_2} and the kinetics of lactic acid movement relative to controls. Initially, Pa_{CO_2} increased above control exercise levels (significant in series C only, Fig. 3) and then declined below that in controls

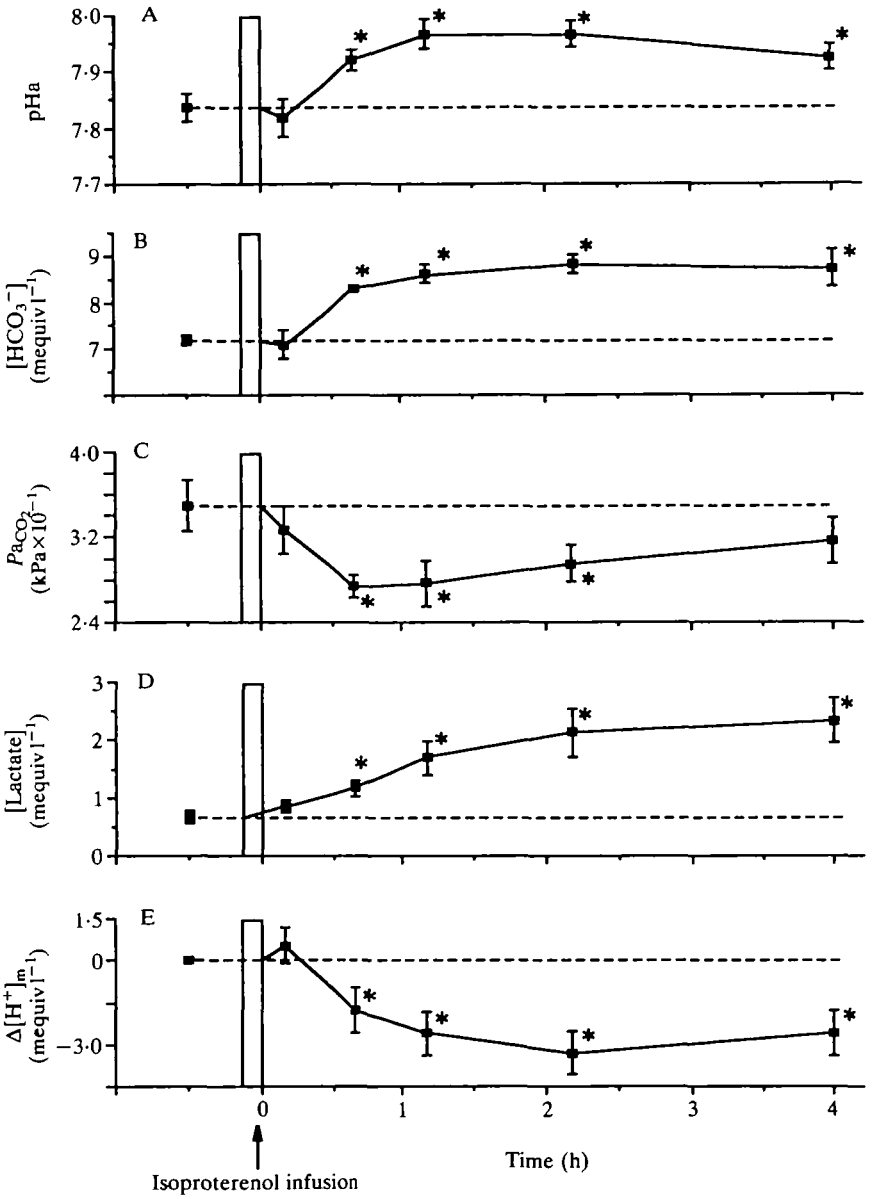

Fig. 1. The effects of isoproterenol infusion (2 μmol kg^{-1}) on (A) arterial blood pH (pHa), (B) whole-blood [bicarbonate] ($[HCO_3^-]$), (C) arterial P_{CO_2} (Pa_{CO_2}), (D) [lactate], and (E) buffered fixed acids ($\Delta[H^+]_m$) in rainbow trout. Means±1 S.E.M. (N=10). Asterisks indicate mean significantly different (P<0.05) from pre-infusion mean (by paired t-test).

and/or propranolol-treated fish (Figs 2, 3). The initial elevation of Pa_{CO_2} may reflect increased titration of HCO_3^- relative to controls (note the higher $\Delta[H^+]$ in isoproterenol-treated fish, Fig. 2D), whereas the subsequent lower Pa_{CO_2} levels

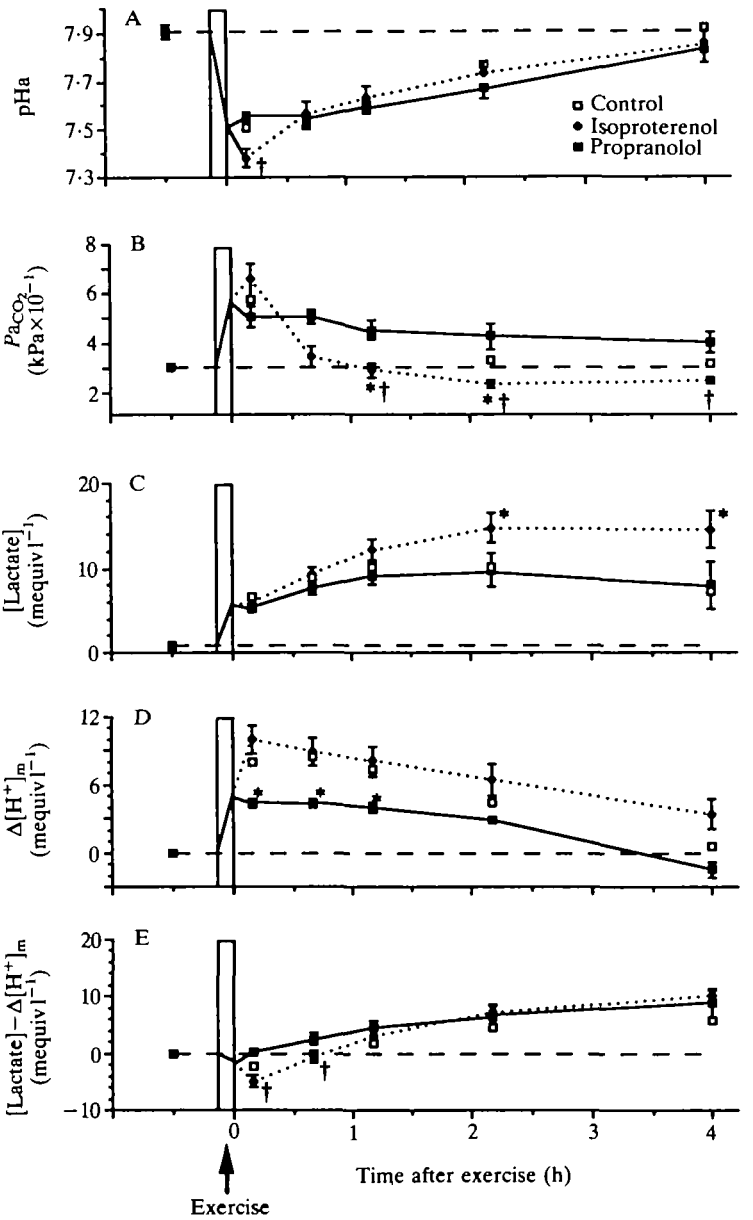

Fig. 2. The effects of exercise and post-exercise isoproterenol (2 μmol kg^{-1}) or propranolol infusion (1.7 μmol kg^{-1}) on (A) arterial blood pH (pHa), (B) arterial P_{CO_2} (Pa_{CO_2}), (C) [lactate], (D) buffered fixed acids ($\Delta[H^+]_m$), and (E) the difference between [lactate] and $\Delta[H^+]_m$ in rainbow trout (series B). Means±1 S.E.M. (N=7 for controls, post-exercise saline infusion; N=10 for post-exercise isoproterenol infusion; N=6 for post-exercise propranolol infusion). S.E. bars have been eliminated from controls for the sake of clarity. Exercise consisted of 10 min of manual chasing; animals were infused immediately following exercise. * Means significantly different (P<0.05) from corresponding value in control animals; † significant differences between isoproterenol- and propranolol-infused fish (by unpaired t-test).

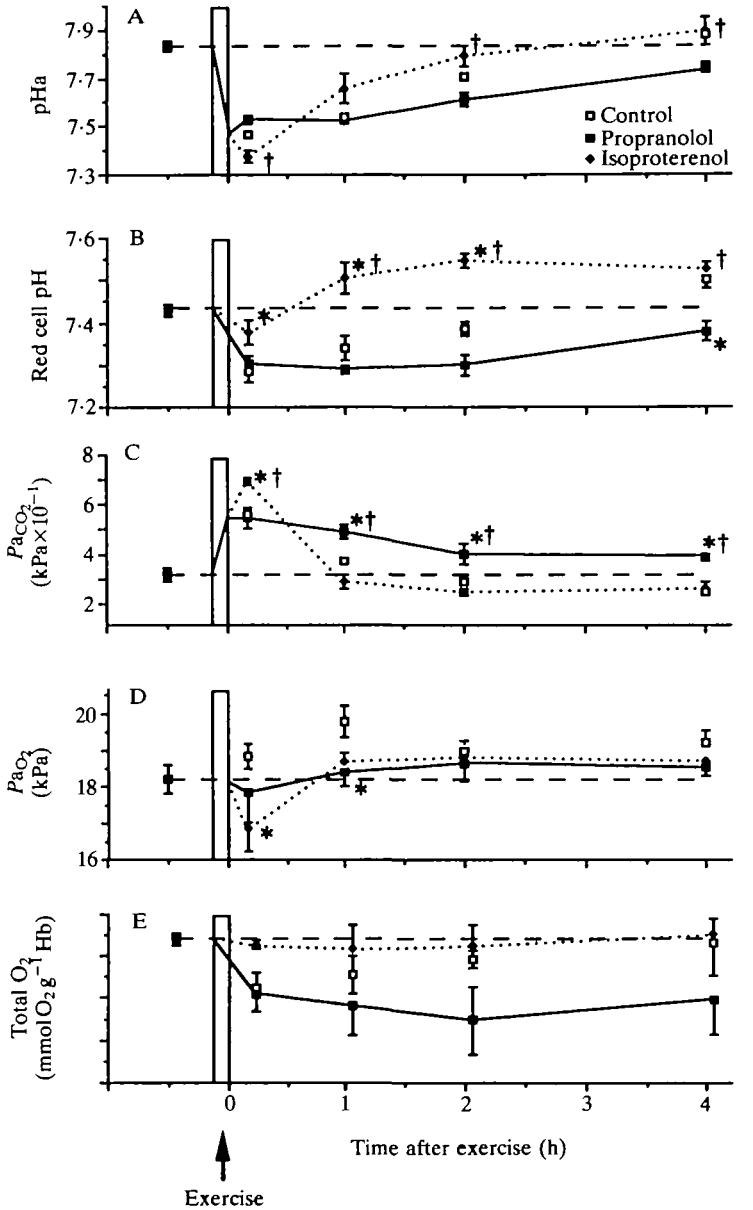

Fig. 3. The effects of exercise and post-exercise isoproterenol (2 μmol kg^{-1}) or propranolol infusion (1.7 μmol kg^{-1}) on (A) arterial blood pH (pHa), (B) red cell pH, (C) arterial P_{CO_2} (Pa_{CO_2}), (D) arterial P_{O_2} (Pa_{O_2}), and (E) O_2 content of haemoglobin (mmol O_2 g^{-1} Hb) in rainbow trout (series C). Means±1 S.E.M. (N=5 in all cases). Exercise consisted of 10 min of manual chasing; animals were infused immediately following exercise. * Means significantly different (P<0.05) from corresponding value in control animals; † significant differences between isoproterenol- and propranolol-infused fish (by unpaired t-test).

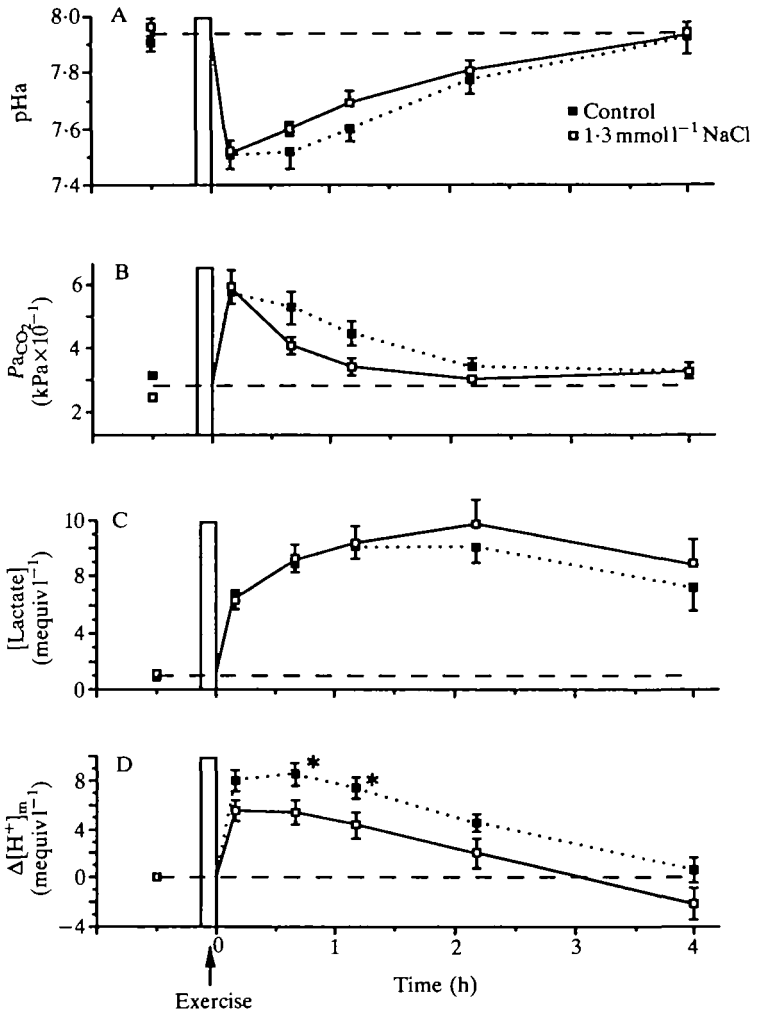

Fig. 4. The effects of exercise and post-exercise elevation of external [NaCl] (from 0.3 to 1.3 mmol l^{-1}) on (A) arterial blood pH (pHa), (B) arterial P_{CO_2} (Pa_{CO_2}), (C) [lactate], and (D) buffered fixed acids ($\Delta[H^+]_m$) in rainbow trout (series D). Means±1 S.E.M. (N=7 for controls, data from Fig. 2; N=9 for 1.3 mmol l^{-1} NaCl). Exercise consisted of 10 min of manual chasing. Asterisks indicate means significantly different (P<0.05) from corresponding value in control animals (unpaired t-test).

probably reflected a β-adrenoreceptor-mediated increase in CO_2 excretion relative to controls. Blood lactate levels progressively increased in isoproterenol-treated fish relative to controls (significantly higher at 2 h and 4 h post-exercise, Fig. 2C). This probably accounts for the higher $\Delta[H^+]_m$ in these animals (Fig. 2D) and suggests that lactic acid release from muscle is sensitive to β-adrenergic regulation. The most dramatic effect of isoproterenol was on red cell pH (Fig. 3B). Here, isoproterenol virtually eliminated the post-exercise acidosis seen

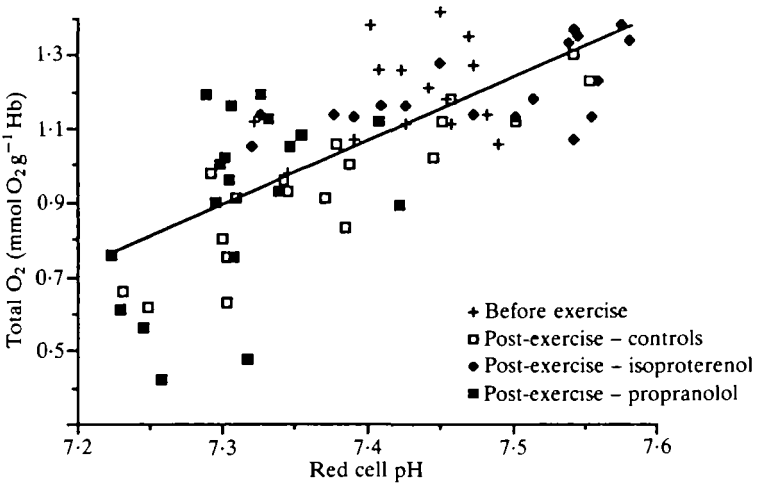

Fig. 5. Relationship between red cell pH and O_2 bound to haemoglobin for arterial blood of rainbow trout (Root effect). All measurements made prior to and following exercise are included except those where P_{O_2} was less than 19 kPa. Data fitted by least-squares regression. Relationship is total $O_2 = -11.5 + 1.7$pHi, $r = 0.731$.

in controls. Red cell pH rose quickly post-exercise to levels above pre-exercise values. This effect on red cell pH had, however, no effect on the oxygen content of arterial blood relative to controls (expressed in terms of mmol O_2 g^{-1} Hb, Fig. 3E), despite an initial significant depression in Pa_{O_2} relative to controls (Fig. 3D).

Generally speaking, propranolol treatment more than reversed the effects of isoproterenol treatment. There was a persistent elevation of Pa_{CO_2} relative to controls and/or isoproterenol-treated fish (Figs 2B, 3C) and significantly reduced $\Delta[H^+]_m$ relative to controls (Fig. 2D). The simplest interpretation of these findings is that propranolol inhibited branchial CO_2 excretion and release of lactic acid from muscle. Furthermore, there was a pronounced red cell acidosis associated with propranolol treatment (Fig. 3B). Although red cell pH in control fish recovered to pre-exercise levels in about 2 h post-exercise, the recovery in propranolol-treated fish was much longer. As a result, oxyhaemoglobin (HbO_2) levels (Fig. 3E) were persistently lower compared with controls and isoproterenol-treated fish.

Post-exercise elevation in external NaCl levels (from 0.3 to 1.3 mmol l^{-1}, series D) had little effect upon blood acid–base status relative to controls (Fig. 4), except for a significantly lower $\Delta[H^+]_m$ (Fig. 4D). This is undoubtedly related to the significantly higher post-exercise net acid excretion (J^H_{net}) relative to controls (see below).

In all fish, red cell pH and HbO_2 were closely correlated. When all data from all treatments prior to and following exercise were combined, the resulting regression equation was: HbO_2 (ml O_2 g^{-1} Hb) $= -11.5 + 1.7$pHi, $r = 0.731$ (Fig. 5). Any blood samples where the Pa_{O_2} was less than 19 kPa were excluded from this regression,

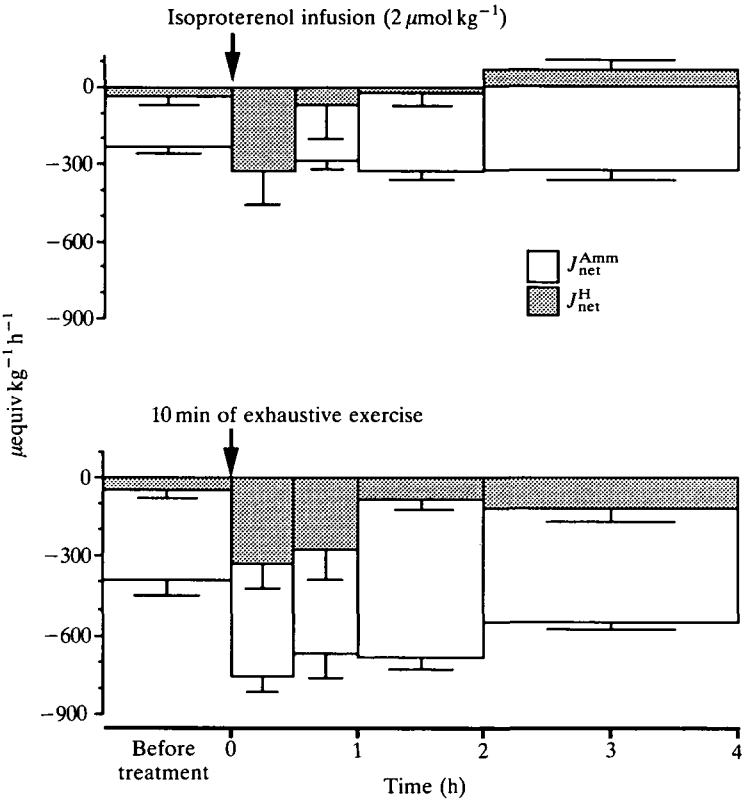

Fig. 6. Ammonia excretion (J^{Amm}_{net}, open bars) and net acid transfer (J^{H}_{net}, shaded bars) before and after isoproterenol infusion (2 μmol kg^{-1}; A, N=10, series A), and following 10 min of exhaustive exercise (N=7, series B). Bars indicate 1 S.E.M.

i.e. samples were excluded where haemoglobin may not have been fully O_2-saturated.

Branchial net acid transfer (series A, B and D)

Effect of isoproterenol infusion

Isoproterenol infusion had, by itself, an immediate, although short-lived, effect upon net acid transfer (J^{H}_{net}) across the gills (Fig. 6A). There was a fairly pronounced net acid excretion over the first 0.5 h after infusion, similar in magnitude to that caused by exhaustive exercise (Fig. 6B) over the same period, but which subsequently declined to values not significantly different from zero. Nonetheless, the quantity excreted over the 0.5 h after infusion, 162 μequiv kg^{-1}, is sufficient to explain the alkalosis which developed in arterial blood (Fig. 1A). The latter is estimated to be 90 μequiv kg^{-1} by 0.5 h after infusion (which is the net alkalosis in extracellular fluids extrapolated from $\Delta[H^+]_m$ estimates in blood according to procedures outlined by McDonald and Wood, 1981).

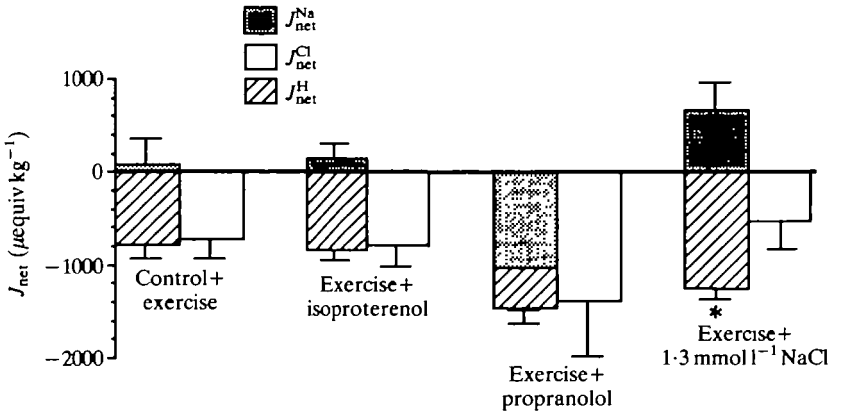

Fig. 7. Net ion and H^+ fluxes (in μequiv kg^{-1}) for 4 h post-exercise. Means+1 S.E.M. [N=7 for control exercise (saline infusion post-exercise); N=10 for isoproterenol infusion, post-exercise, series B; N=6 for propranolol infusion post-exercise; and N=9 for exercise+1.3 mmol l^{-1} NaCl, series D]. Asterisk indicates J^{H}_{net} significantly different from control+exercise.

Effects of exercise

The post-exercise net acid excretion (Fig. 6B), while similar over the first 0.5 h to that induced by isoproterenol infusion, was overall larger, amounting to a total of 717±125 μequiv kg^{-1} (mean±1 S.E.M., N=11) by 4 h post-infusion (Fig. 7). This amount is similar to that previously reported for exhaustively exercised rainbow trout (≈1000 μequiv kg^{-1}; Wood, 1988), and the overall pattern of excretion is also similar (i.e. significant stimulation of ammonia excretion, net acid excretion highest immediately post-exercise and subsequently declining; Fig. 6B).

Isoproterenol infusion, despite having significant effects on blood gases and acid–base status of exercised fish and causing significant net acid excretion in non-exercised fish, did not further increase net acid transfer beyond that of control exercise (Fig. 7). In contrast, post-exercise propranolol infusion reduced net acid excretion by about 60 % of control exercise values. However, the effect was quite variable and, as a result, not significant. The only significant effect upon post-exercise J^{H}_{net} was that resulting from elevating external [NaCl] from 0.3 to 1.3 mmol l^{-1} (Fig. 7). Here, net acid excretion over 4 h was increased by 78 % to 1278±112 μequiv kg^{-1} (N=9). This clearly establishes external NaCl as a limiting factor in net acid transfer across the gills of freshwater fish.

Interrelationship between ion and acid–base fluxes

Since net acid transfer across the gills is a transfer of electrical charge, charge balance must be met by the movement of one or more of the so-called 'strong' ions (i.e. any ion that does not have acid–base-relevant status). As Fig. 7 illustrates, the important 'strong' ions in this charge balance are Na^+ and Cl^-. Indeed, in all

treatments the post-exercise fluxes very closely approximated the following equation:

$$J^{H}_{net} = J^{Cl}_{net} - J^{Na}_{net}\,, \tag{5}$$

where a negative value means net ion or acid loss and a positive value means net ion or acid uptake (or base loss). Any discrepancies (attributable to errors in measurement of $[Na^+]$, $[Cl^-]$, [ammonia] and TA in water and/or to the fluxes of unmeasured 'strong' ions such as Ca^{2+} and K^+) were quite small and thus could be ignored.

Since the fluxes of Na^+ and Cl^- consist of both influx and efflux components, equation 5 can be rewritten as follows:

$$J^{H}_{net} = J^{Cl}_{in} - J^{Cl}_{out} - (J^{Na}_{in} - J^{Na}_{out})\,, \tag{6}$$

or by rearrangement:

$$J^{H}_{net} = (J^{Cl}_{in} - J^{Na}_{in}) + (J^{Na}_{out} - J^{Cl}_{out}) \tag{7}$$

$$= \text{influx component} + \text{efflux component}\,.$$

Branchial net acid transfer can thus be resolved into two components; one arising because of a difference between the rates of active uptake of Na^+ and Cl^- (the influx component), and the other from a difference between the rates of diffusional efflux of Na^+ and Cl^- (the efflux component).

Fig. 8 shows the influx and efflux components of J^{H}_{net} for the various exercise protocols calculated from unidirectional ion flux rates according to equation 7. For all exercise protocols there was a significant stimulation of the influx component, which, by itself, would have resulted in a net acid excretion. For exercise where external NaCl was $0.3\,mmol\,l^{-1}$ (Fig. 8A,B,C), the influx component was fairly uniform at approximately $400\,\mu equiv\,kg^{-1}\,h^{-1}$ over the first 2 h after exercise, whereas at $1.3\,mmol\,l^{-1}$ NaCl it was more than double that over the same period (Fig. 8D). In all instances the influx component arose entirely from a stimulation of Na^+ influx (Table 1). There was no significant change, either an increase or a decrease, in J^{Cl}_{in} in any case, not even when external Cl^- levels were increased more than four times (from 0.3 to $1.3\,mmol\,l^{-1}$, Fig. 8D). Isoproterenol infusion (without exercise) evoked a similar increase in Na^+ influx to that resulting from exercise (Table 1). However, J^{Na}_{in} was not further stimulated by isoproterenol infusion when combined with exercise, nor was it inhibited by propranolol infusion after exercise (Table 1).

The efflux component (Fig. 8, shaded bars) was typically of opposite sign to the influx component; i.e. by itself it would have led to net acid uptake rather than excretion. This component was particularly elevated following propranolol infusion (Fig. 8C) and in response to the increased external [NaCl] (Fig. 8D). The efflux component arose because J^{Na}_{out} increased more than J^{Cl}_{out} after exercise (Table 1).

In contrast to influx, where only J^{Na}_{in} was elevated, exercise significantly stimulated (by at least threefold) both Na^+ and Cl^- efflux. This is probably the

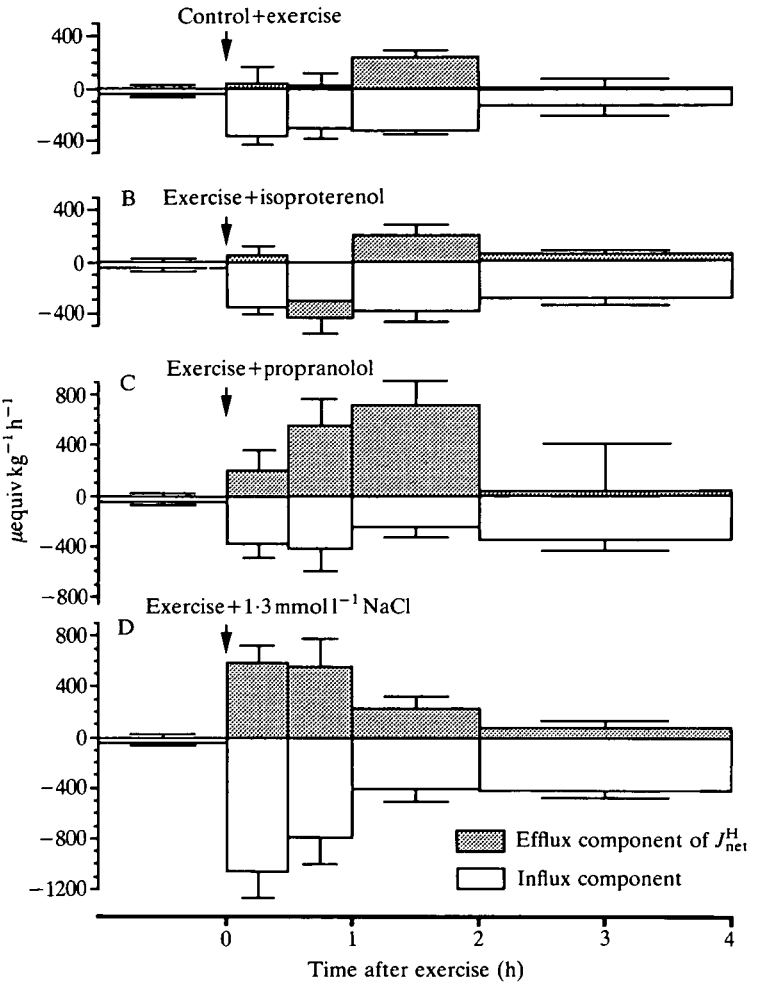

Fig. 8. Influx ($J^{Cl}_{in}-J^{Na}_{in}$) and efflux ($J^{Na}_{out}-J^{Cl}_{out}$) components of J^{H}_{net} following control exercise (A, $N=7$, series B), exercise+isoproterenol infusion (B, $N=10$, series B), exercise+propranolol infusion (C, $N=6$, series B), and exercise+1.3 mmol l^{-1} NaCl (D, $N=9$, series D). Data are means+1 S.E.M. See text for further details.

result of the well-known increase in branchial ion and water permeability that accompanies exercise in freshwater fish (Wood and Randall, 1973). In contrast, the increase in branchial ionic efflux following isoproterenol infusion (without exercise) was smaller and more variable and, consequently not statistically significant (Table 1).

Discussion

The present study confirms and extends previous studies by showing an important role for catecholamines in the recovery of metabolic and acid–base

Table 1. *Influx and efflux of Na^+ and Cl^- (in μequiv kg^{-1} h^{-1}) prior to and immediately post-treatment*

			Exercise			
	Pre-treatment	Isoproterenol infusion	Control	Isoproterenol infusion	Propranolol infusion	1.3 mmol l^{-1} NaCl
J_{in}^{Na}	311±18	488±63	557±57*	514±65*	652±92*	1288±220*
J_{in}^{Cl}	219±14	217±25	191±24	171±35	275±34	234±64
J_{out}^{Na}	205±25	383±153	611±140*	824±103*	918±128*	1349±221*
J_{out}^{Cl}	215±26	436±122	569±121*	776±120*	708±211*	766±179*
Duration (h)	1	0.5	0.5	0.5	0.5	0.5
N	41	10	7	10	6	9

* Significantly different from pre-treatment value by paired *t*-test ($P<0.05$) using each fish as its own control.

Note the pre-treatment mean is the combined data for all fish.

Pre-treatment means from individual treatments were not significantly different from one another.

Values are means±S.E.M.

homeostasis following exhaustive exercise. It is now well known that catecholamines are elevated in exercise; up to 69-fold increases in levels of adrenaline and 15-fold increases in noradrenaline (above resting levels of 2–3 nmol l^{-1}) have been reported in the freshwater-adapted rainbow trout exercised to exhaustion (see Tang and Boutilier, 1988, for a recent review). We show that β-adrenoreceptors are the key to this recovery process by the way that propranolol prolonged and isoproterenol promoted recovery. Furthermore, our finding of a significant effect of isoproterenol indicates that endogenous levels of catecholamines were not sufficient to have maximally stimulated β-adrenergic receptors. In this study we can identify four specific, separate targets for β-adrenergic regulation: branchial gas exchange, red cell oxygenation and acid–base status, lactic acid kinetics, and branchial ion and acid fluxes.

Branchial gas exchange

Catecholamines are thought to influence gas transfer in fish in at least two ways: through a specific, β-adrenoreceptor-mediated increase in ventilation (Peyraud-Waitzenegger, 1979) and through an increase in branchial gas transfer capacity (Randall and Daxboeck, 1984). The latter probably results from a combination of a direct β-adrenoreceptor-mediated effect on permeability of the epithelium (Isaia *et al.* 1978) and a vasomotor effect on functional surface area (increased cardiac output and blood pressure, β-adrenoreceptor-mediated branchial vasodilation, lamellar recruitment, redistribution of blood flow in the gills; Randall and Daxboeck, 1984). We confirm that β-adrenergic receptors appear to be dominant in this effect. Bolus isoproterenol infusion into either normoxic resting fish (Fig. 1C) or exercised fish (Figs 2B, 3C) caused a significant and prolonged

reduction in Pa_{CO_2}, although in the latter there was an initial post-exercise rise in Pa_{CO_2}. Furthermore, β-adrenoreceptor blockade reversed the effects of β-stimulation; propranolol infusion following exercise significantly increased Pa_{CO_2} relative to values in either isoproterenol-treated or exercised but untreated control fish (Figs 2B, 3C).

The rise in Pa_{CO_2} following exercise (Figs 2, 3) is commonly observed in teleost fish (Wood and Perry, 1985), though its underlying cause is under some dispute. Wood and Perry (1985) have argued that catecholamines released during exhaustive exercise effectively inhibit bicarbonate ion movements through the erythrocyte membrane, thereby leading to an overall retention of CO_2. If this occurs, it should, in turn, lead to a reduction in CO_2 excretion following either exhaustive exercise or catecholamine infusion. However, experiments of this sort, carried out on rainbow trout (Steffenson *et al.* 1987; Tufts *et al.* 1988) or coho salmon (Milligan and McDonald, 1988) did not show any appreciable reduction in CO_2 excretion. Thus, it would appear that the dominant effect of catecholamines is to promote CO_2 excretion, presumably through cardiorespiratory adjustments at the gills (Randall and Daxboeck, 1984), whatever their specific effects on bicarbonate permeability of red cells.

Red cell oxygenation and acid–base status

Probably the most pronounced (and most easily reproducible) effect of circulating catecholamines is the β-adrenergic regulation of red cell pH, an effect that helps to sustain blood oxygen-transport in the face of extracellular acidosis (Boutilier *et al.* 1986; Primmett *et al.* 1986; Milligan and Wood, 1987). We confirm this finding by showing that propranolol caused a drop and isoproterenol an increase in red cell pH relative to exercised but untreated controls (Fig. 3B). There were corresponding effects on red cell oxygenation, predictable on the basis of a Root effect on haemoglobin (Fig. 5). We can now report two observations different from those given in previous studies. The first is the significant post-exercise reduction in red cell pH in control fish. This contrasts with earlier findings that red cell pH was maintained relatively constant in the rainbow trout in the face of extracellular acidification (caused either by exhaustive exercise; Primmett *et al.* 1986; Milligan and Wood, 1987; or by mineral acid infusion; Boutilier *et al.* 1986). The implications of this finding are unclear but the difference could be because of the lower catecholamine mobilization in this study. Nonetheless, the fall in intracellular pH (pHi) was less than would have been expected from acid titration *in vitro* with catecholamines absent (Boutilier *et al.* 1986). Second, the Root effect we report is almost double that determined *in vitro* in the rainbow trout over a similar pH range (pH 7.3–7.7; Boutilier *et al.* 1986). The latter was determined with catecholamines at resting levels ($1\,nmol\,l^{-1}$). Therefore, the elevation of catecholamine levels *in vivo* may act to increase the magnitude of the Root effect and further benefit blood O_2 transport. This implies that catecholamines have a direct effect on O_2 binding by haemoglobin separate from that of pHi. We are not suggesting that catecholamines traverse the red blood cell membrane but rather

that they may act through their well-known effect on ATP metabolism (Ferguson and Boutilier, 1989) to produce such changes in haemoglobin oxygenation.

Lactic acid kinetics

One of the valuable features of exhaustive exercise as an experimental tool is that lactic acid production is really quite uniform from one individual to the next (Milligan and Wood, 1986*a*,*b*). Further, since drugs were infused *after* exercise it is reasonable to assume that differences in levels of blood lactate amongst treatment groups resulted from effects on lactic acid *kinetics* rather than *production*. Significantly higher blood lactate levels in isoproterenol-treated fish (Fig. 2C) and significantly lower $\Delta[H^+]_m$ (metabolic acid levels) in propranolol-treated fish (Fig. 2D) suggest that the removal of lactic acid from white muscle is, at least partially, under β-adrenergic control. This notion is further supported by the finding that isoproterenol infusion also significantly elevated blood lactate levels in resting, normoxic fish (Fig. 1D). In these fish there is normally a large electrochemical gradient favouring lactate efflux from muscle. Intracellular lactate levels are either similar (Y. Tang, unpublished results) or several-fold higher than blood levels (Milligan and Wood, 1986*b*), and white muscle membrane potentials are approximately $-80\,mV$, inside negative (Hagiwara and Takahashi, 1967). Catecholamines could promote increased lactate release by increasing muscle perfusion through β-mediated vasodilation (Wood, 1976), but direct effects upon membranes are probably more important since Neumann *et al.* (1983) have shown that removal of lactate from muscle is diffusion- rather than perfusion-limited.

Catecholamines could act directly on membranes either by increasing lactate permeability or by blocking an active inward carrier, as suggested earlier by Turner and Wood (1983). As evidence, these authors showed a threefold increase in lactate efflux from trout white muscle following administration of SITS, an anion channel blocker. Nonetheless, the picture is far from straightforward. Wardle (1978) showed that the β-agonist isoxsuprine had exactly the opposite effect on the exercised plaice, *Pleuronectes platessa*, as in the present study; that is, it resulted in a retention of lactate in the muscle while propranolol led to increased release. Wood and Milligan (1987) were unable to show any β-mediated effect on lactate kinetics in the related flatfish, *Platichthys stellatus*, nor were van Dijk and Wood (1988) able to show an effect of propranolol on post-exercise lactic acid kinetics of freshwater rainbow trout. The latter used a fivefold higher dose than in the present study, but an otherwise similar experimental protocol, and suggested the lack of effect may be ascribable to seasonal differences in β-receptor population density. Thus, a role for β-adrenoreceptors in the movement of lactate remains an intriguing possibility, but one requiring further work to resolve.

Branchial ion and H^+ fluxes

Based largely on the results of perfused trout head studies, Wood and Perry (1985) proposed that endogenous catecholamines controlled branchial acid transfers by manipulating Na^+/H^+ and Cl^-/HCO_3^- exchanges at the gills;

Na^+/H^+ exchange activated by β-receptors and Cl^-/HCO_3^- exchange by α-receptors. More recently, Vermette and Perry (1987) showed that continuous epinephrine infusion elicited a net branchial acid excretion in rainbow trout. However, the infusion also caused an extracellular acidosis; i.e. the acidosis rather than the epinephrine infusion may have been the proximate stimulus to net acid excretion. Our result with isoproterenol is, therefore, much more straightforward. Bolus infusion provoked a rapid alkalosis due entirely to net acid excretion at the gills which, in turn, appeared to arise solely as a result of stimulation of Na^+ influx (i.e. Na^+/H^+ exchange). Exercise provoked a similar initial stimulation of Na^+ influx and H^+ excretion, which was more sustained, in proportion to the blood acid–base disturbance. Isoproterenol infusion, in this instance however, had no effect, suggesting that the branchial response may already be maximal as a consequence of exercise. Any further increase appears to depend on the availability of the counterion for Na^+/H^+ exchange, as indicated by the stimulation in acid excretion resulting from the increase in external $[Na^+]$ (Figs 7, 8).

However, one complicating feature in this otherwise simple picture is the complete failure of propranolol to block either the Na^+ influx increase of exercise (Table 1) or, for that matter, the influx component of acid excretion (Fig. 8). In fact, Na^+ influx following propranolol infusion was slightly greater than for either control+exercise or isoproterenol+exercise (Table 1). This suggests that other endogenous factor(s) are responsible for the post-exercise increase in Na^+ influx. Possible candidates include alpha effects of circulating catecholamines, central activation of adrenergic and cholinergic neurones, and other putative humoral agents (e.g. glucagon, vasoactive intestinal peptide, prolactin, cortisol, urotensin, atrial naturetic factor). The participation of these factors in the regulation of acid–base-relevant ion transfers in freshwater fish is a fruitful area for future research.

In addition to the influx component, a number of recent studies have shown that there can also be a significant contribution by the efflux component to net acid transfer (Vermette and Perry, 1987; McDonald and Prior, 1988; Wood, 1988; McDonald *et al.* 1989). The efflux component can amount to as much as one-third of the total in the case of correction of acidosis due to ammonium sulphate infusion (McDonald and Prior, 1988) or the correction of alkaloses arising from either bicarbonate infusion or prolonged hyperoxia (McDonald *et al.* 1989). In contrast, the present study shows no contribution by the efflux component to the correction of the acidosis. Rather, it tended to cancel out the influx component, particularly in the case of propranolol treatment, since it was of opposite sign. This makes little sense as a regulatory strategy and our view is that the efflux component is not, in fact, subject to regulation but rather reflects an intrinsic property of the gills. We feel this arises from two phenomena: (1) branchial ionic permeability unavoidably increases as a consequence of the haemodynamic changes of exercise (Wood and Randall, 1973), and (2) the branchial diffusion channels responsible for electrolyte efflux are intrinsically more permeable to Na^+ than to Cl^- (McDonald *et al.* 1983).

Thus, with exercise, Na^+ efflux should increase more than Cl^- efflux (Table 1) and this, by strong ion difference theory, would cause the net loss of *basic* equivalents (providing, of course, that no other strong ions are available to balance the charge).

This intrinsic property and the supply of external NaCl probably contribute to limiting net acid transfer by the gills of freshwater fish. While it cannot be stated with any certainty which factor is primary in setting the limit, it is worth noting that with an identical exercise protocol, the seawater-adapted rainbow trout excreted more than twice as many acidic equivalents as its freshwater counterpart (Tang *et al.* 1989). Here, external NaCl levels were much higher and permeability characteristics of the gills fundamentally different. Thus, either factor could act to limit net acid transfer.

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