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THE EFFECT OF PERFUSION FLOW RATE AND ADRENERGIC STIMULATION ON OXYGEN TRANSFER IN THE ISOLATED, SALINE-PERFUSED HEAD OF RAINBOW TROUT (*SALMO GAIRDNERI*)

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SUMMARY

An isolated, saline-perfused trout head preparation, irrigated with hyperoxic water ($Pw_{O_2} = 250$ Torr), was used to assess diffusion/perfusion limitations to gill oxygen transfer. In the absence of catecholamines, increasing the perfusion flow rate caused a reduction of the partial pressure of oxygen in the dorsal aortic perfusate, indicating diffusion limitations to oxygen uptake. Physiological concentrations of epinephrine stimulated oxygen uptake in a dose-dependent fashion. Moreover, epinephrine elicited a greater effect during increased perfusion flow rate as a result of larger initial diffusion limitations, caused by the increased flow. By using a variety of adrenergic agonists and antagonists, it was demonstrated that beta-receptor stimulation enhanced oxygen uptake whereas alpha-receptor stimulation had no effect. These results are discussed with reference to changes in gill epithelial permeability to oxygen and/or surface area changes.

INTRODUCTION

There is considerable controversy about whether oxygen transfer across fish gills is perfusion or diffusion limited. Results of recent studies using isolated, saline-perfused head preparations (Pettersson & Johansen, 1982; Pärt, Tuurala & Soivio, 1982; Pettersson, 1983) have shown that epinephrine enhances gill oxygen transfer. These observations have confirmed earlier speculation that *in vivo* injections of epinephrine in the eel (*Anguilla anguilla*) elevate arterial P_{O_2} (Pa_{O_2}) by increasing gill O_2 diffusing capacity (Steen & Kruysse, 1964; Peyraud-Waitzenegger, 1979). The effect of epinephrine on gill O_2 diffusing capacity is due to increased functional surface area (Booth, 1979) and/or increased epithelial permeability to oxygen (Pettersson, 1983). These results suggest that the fish gill is diffusion limited with respect to O_2 transfer.

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A similar conclusion was reached by Fisher, Coburn & Forster (1969) from studies on carbon monoxide transfer across the gills of the bullhead catfish (*Ameiurus nebulosus*). Another method for investigating diffusion/perfusion limitations in gills is to alter flow ($\dot{Q}$in) in perfused preparations while monitoring Pa_{O_2}. With this approach, Daxboeck, Davie, Perry & Randall (1982), using a spontaneously ventilating, blood-perfused trout preparation, and P. Pärt (personal communication), using a saline-perfused trout head preparation, have concluded that fish gills are primarily perfusion limited with respect to O_2 transfer. This conclusion was based on the observation that increasing $\dot{Q}$in did not reduce Pa_{O_2}. These results may not be representative of the situation in intact, resting fish because of the high concentration of epinephrine employed by Pärt (10^{-6} mol l^{-1}) and the probability of high levels of circulating catecholamines in the highly-stressed, blood-perfused trout preparation (experiments commenced following only 2–3 h of recovery from extensive surgery). In other words, it is not surprising that the gills are perfusion limited under these conditions, considering the known effect of epinephrine on increasing gill O_2 diffusing capacity.

In this study, we have used a novel approach whereby the gills were irrigated with hyperoxic water ($Pw_{O_2} \simeq 250$ Torr) in order to obtain a stable, saline-perfused preparation in the absence of catecholamines. With this technique we have attempted to clarify the diffusion/perfusion limitations in perfused fish gills by modifying $\dot{Q}$ and monitoring Pa_{O_2}. Moreover, we have investigated the action of epinephrine on gill O_2 transfer both at 'normal' $\dot{Q}$ and elevated $\dot{Q}$ to test the hypothesis that epinephrine has a greater effect at high flow rates, due to increased diffusion limitations. Additionally, we have investigated the nature of the adrenergic receptors which control oxygen transfer by perfusing with a variety of alpha- and beta-agonists and antagonists.

MATERIALS AND METHODS

Adult rainbow trout (*Salmo gairdneri* Richardson) of either sex, weighing between 200 and 300 g, were obtained from Sun Valley Trout Farm (Mission, BC). They were kept outdoors in large, circular fibreglass tanks and supplied with aerated, dechlorinated Vancouver tap water ($Na^+ = 40$ μequiv l^{-1}, $Cl^- = 20$ μequiv l^{-1}, hardness = 12 p.p.m. $CaCO_3$), at ambient temperature and photoperiod. Fish were fed daily with a commercial trout diet (Moore-Clark Co.). They were not fed for 48 h prior to experimentation.

Isolated, saline-perfused head preparations were obtained according to Payan & Matty (1975) with minor modifications. Fish were injected with sodium heparin (2500 USP units $fish^{-1}$) either directly into the caudal vein/artery or intraperitoneally. Following a 20 min period, the fish was decapitated just behind the pectoral fins. The head was placed onto an operating table and the gills were irrigated with water *via* a tube placed into the mouth. As quickly as possible the pericardium was cut and the ventricle severed. Next, the head was pithed by inserting a metal probe along the neural arch into the cranium (Oduleye, Claiborne & Evans, 1982; S. F. Perry & C. Daxboeck, in preparation). Pithing has been shown to cause no adverse effects on

gill oxygen transfer or haemodynamics (S. F. Perry & C. Daxboeck, in preparation) and simplifies surgical procedures by eliminating head movement. Viscera were removed from the body cavity and a catheter (Clay, Adams, PE 90) was inserted into the bulbus arteriosus through the severed ventricle and tied in place. The gills were cleared of blood by perfusing with filtered (0·45 μm, Millipore) saline (Payan & Matty, 1975; HCO_3^- concentration was changed from 26 to 13 mmol l^{-1}, a concentration more representative of *in vivo* values Perry, Haswell, Randall & Farrell, 1981) at a constant pressure of 50 cmH_2O (Perry, Payan & Girard, 1984). A tightly fitting semi-circular plastic collar was placed into the abdominal cavity and sutured into position, thus making the body wall rigid. Finally, a catheter (Clay Adams, PE 200) was inserted into the dorsal aorta for collection of post-branchial saline. On average, the surgery was completed in approximately 8 min and the gills were ischaemic for periods never exceeding 2 min. At this stage, the gills were inspected visually to determine efficiency of clearing. Gills which displayed less than approximately 80 % clearance of blood cells were discarded (less than 10 % of all preparations).

The head was removed from the operating table and placed into a cylindrical plastic container and held in place by a thin rubber membrane which prevented leakage of the recirculating fresh water contained inside. The head was irrigated at about 500 ml min^{-1} with aerated (mean $Pw_{O_2} = 142$ Torr $\pm$ 1·1 S.E.) or hyperoxic (mean $Pw_{O_2} = 253$ Torr $\pm$1·4 S.E.) dechlorinated tap water (maintained at 9°C) by a tube placed into the mouth. The gills were perfused using constant pulsatile flow ($\sim$ 1·5 ml min^{-1} 100 g^{-1} body weight; pump frequency = 40 strokes min^{-1}) from a double reservoir, with gas-equilibrated saline (0·3 % CO_2, 5 % O_2, remainder N_2; $P_{CO_2} = 2{\cdot}3$ Torr, $P_{O_2} = 40$ Torr) using a cardiac pump (Harvard, model 1405) as modified according to Davie & Daxboeck (1983). Dorsal aortic pressure (Pda) was kept at zero in all experiments. Gas mixtures were supplied by gas mixing pumps (Wösthoff). Input pressure (Pin) was monitored from a T-junction in the input catheter connected to a Statham P23Db pressure transducer and displayed on a chart recorder (Gould). Catheter resistance was measured upon termination of each experiment and Pin was corrected accordingly. Isolated heads which exhibited Pin greater than 80 cmH_2O were indicative of trapped air bubbles in the gill vasculature and were discarded (approximately 10 % of all preparations). Heads were perfused for a period of 10–15 min (or until Pin had stabilized) before experiments were started (time = zero). All experiments commenced with zero catecholamine content in the perfusate except where otherwise stated.

Protocol

Four separate series of experiments were performed. In the first series, a comparison of oxygen transfer and haemodynamic variables was made between heads perfused with saline containing 10^{-7} mol l^{-1} norepinephrine and irrigated with normoxic water ($Pw_{O_2} = 142$ Torr) and heads perfused without norepinephrine but irrigated with hyperoxic water ($Pw_{O_2} = 253$ Torr). The objective of these comparisons was to try and develop a relatively stable short-term preparation in the absence of high levels of catecholamines such that subsequent manipulations of perfusion flow rate

and adrenergic stimulation could be performed without catecholamines in the perfusate. Indeed, all further experiments were performed using hyperoxic water. The second series consisted of manipulations of the perfusion flow rate ($\dot{Q}$), to determine what effects these had on Pa_{O_2}, oxygen uptake ($\dot{M}_{O_2}$), Pin, branchial vascular resistance to flow (Rbv) and perfusate flow distribution ($\dot{Q}in = \dot{Q}da + \dot{Q}av$; where $\dot{Q}in$ is input perfusion flow rate set by the pulsatile pump, $\dot{Q}da$ is dorsal aortic (arterial) perfusate flow and $\dot{Q}av$ is venous drainage from the general head circulation as well as venous outflow from the central filamental sinus of gill filaments). $\dot{Q}in$ was adjusted by varying stroke volume while leaving pumping frequency constant. The third series of experiments was designed to study the effect of epinephrine (10^{-6} and 10^{-7} mol l^{-1}) on Pa_{O_2}, $\dot{M}_{O_2}$, Pin, Rbv and perfusate flow distribution. These effects were documented for preparations perfused at 'normal' flow rates (approximately 4 ml min^{-1}) and at increased flow rate (approximately 6 ml min^{-1}). The final series of experiments was designed to determine the nature of the receptor(s) (alpha or beta) responsible for the stimulatory effect of epinephrine on gill O_2 transfer. Experiments consisted of monitoring Pa_{O_2}, $\dot{M}_{O_2}$, Pin, Rbv and perfusate flow distribution when isoproterenol (isoprenaline, beta agonist), phenylephrine (alpha agonist) and propranolol (beta antagonist) were added to final concentrations of 10^{-6} mmol l^{-1} in the perfusate.

P_{O_2} was determined on samples withdrawn directly from the dorsal aortic catheter (Pa_{O_2}), perfusate reservoir (Pv_{O_2}) or head chamber (Pw_{O_2}) using a Radiometer PHM-71 digital acid-base analyser and associated O_2 electrode at 9°C. $\dot{Q}da$ and $\dot{Q}av$ were determined volumetrically while $\dot{Q}in$ was checked at the beginning and conclusion of each experiment. $\dot{M}_{O_2}$ (μmol min^{-1} 100 g^{-1}) and gill O_2 extraction effectiveness (O_2 ext. eff., %) were calculated using the following equations:

$$\dot{M}_{O_2} = \alpha(Pa_{O_2} - Pv_{O_2}) \times \dot{Q}in\ (l\,min^{-1}) \times 100/\text{body weight (g)}, \quad (1)$$

where α is the solubility coefficient of O_2 as determined for flounder plasma at 9°C (2·05 μmol l^{-1} $Torr^{-1}$; Wood, McMahon & McDonald, 1979) and Pv_{O_2} is the oxygen tension of the inflow (venous) perfusate.

$$O_2 \text{ ext. eff.} = \frac{Pa_{O_2} - Pv_{O_2}}{Pw_{O_2} - Pv_{O_2}} \times 100. \quad (2)$$

Data in tables and figures are presented as means ±1 S.E. Where appropriate, paired or unpaired Student's t-tests were used to compare sample means. Statistical analysis of percentage changes was done following conversion of data using arcsine transformation (Zar, 1974).

RESULTS

A comparison of oxygen transfer and other perfusion variables in heads perfused with 10^{-7} mol l^{-1} norepinephrine under conditions of external normoxia (Pw_{O_2}

= 140 Torr) with heads perfused without any catecholamine under conditions of external hyperoxia (Pw_{O_2} = 250 Torr) is shown in Table 1. Not only were Pa_{O_2} and $\dot{M}_{O_2}$ increased in hyperoxic preparations, as one would predict, but so was the overall stability. In normoxic heads (with 10^{-7} mol l^{-1} norepinephrine) there was considerable deterioration after 20 min as indicated by significant increases of Pin and Rbv as well as significant decreases in Pa_{O_2} and $Pa_{O_2}-Pv_{O_2}$. In contrast, there were no significant changes in any perfusion variable after 20 min during conditions of external hyperoxia. It is important to note that at the onset of perfusion, all haemodynamic variables ($\dot{Q}$da, $\dot{Q}$av, Pin, Rbv) were similar in both types of preparations. The stabilizing effect of external hyperoxia on Pin, in the absence of norepinephrine, is illustrated in Fig. 1. During 30 min perfusion with normoxic water (zero norepinephrine), Pin increased by approximately 15 cmH_2O. In contrast, perfusing under conditions of external hyperoxia resulted in constant Pin throughout the 30-min period.

Increasing perfusion flow rate from the 'normal' rate of 3·84 ml min^{-1} to 5·59 ml min^{-1} resulted in significant reductions in Pa_{O_2} and $Pa_{O_2}-Pv_{O_2}$ (Table 2). $\dot{M}_{O_2}$ was elevated (although not significantly) simply due to the increased flow rate. Input

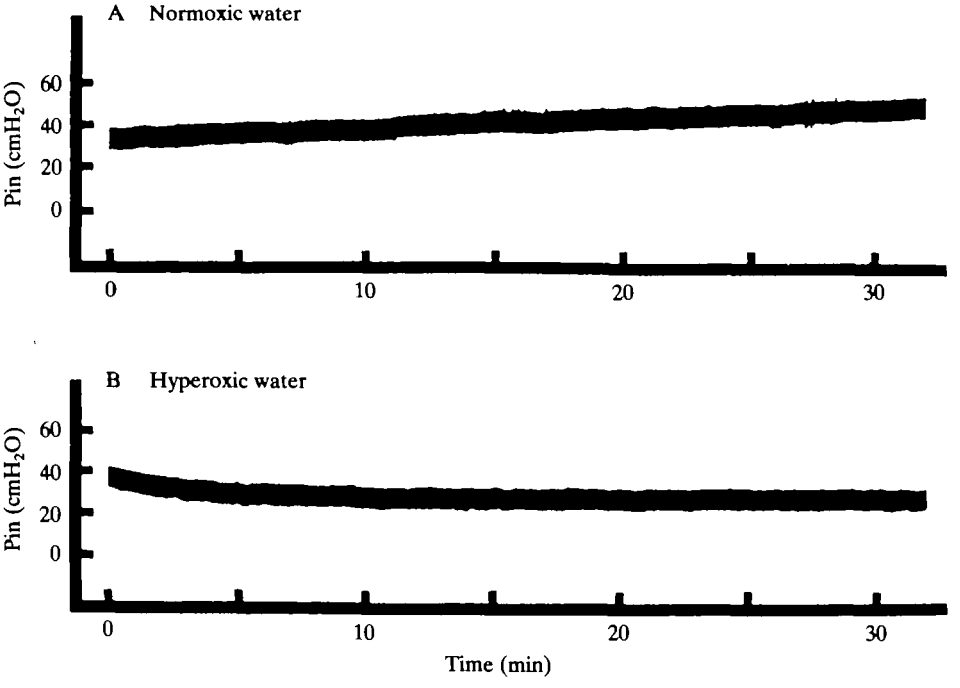

Fig. 1. Input pressure (Pin) in representative isolated, saline-perfused heads of rainbow trout irrigated with (A) normoxic water (Pw_{O_2} = 140 Torr) and (B) hyperoxic water (Pw_{O_2} = 250 Torr). In both cases, perfusate contains zero catecholamine content.

Table 1. *The effect of external hyperoxia on oxygen transfer and haemodynamic variables in the isolated, saline-perfused head of rainbow trout*

Time (min)	$\dot{Q}$in (ml min^{-1})	$\dot{Q}$da (ml min^{-1})	$\dot{Q}$av (ml min^{-1})	Pa_{O_2} (Torr)	$Pa_{O_2}-Pv_{O_2}$ (Torr)	$\dot{M}_{O_2}$ (μmol min^{-1} 100 g^{-1})	Pin (cmH_2O)	Rbv (cmH_2O ml^{-1} min^{-1} 100 g^{-1})
				Normoxia (Pw_{O_2} = 140 Torr; 10^{-7} mol l^{-1} norepinephrine; N = 6)				
0	3·85 ± 0·07	3·49 ± 0·07	0·36 ± 0·04	101·5 ± 5·2	63·7 ± 4·4	0·22 ± 0·02	32·5 ± 2·0	19·1 ± 1·2
10	3·84 ± 0·07	3·45 ± 0·04	0·39 ± 0·05	99·0 ± 5·9	61·5 ± 5·0	0·21 ± 0·02	34·0 ± 2·2*	20·1 ± 1·4*
20	3·82 ± 0·07	3·37 ± 0·08	0·45 ± 0·04	92·5 ± 7·5*	54·5 ± 6·9*	0·19 ± 0·03	37·8 ± 3·0*	22·3 ± 1·7†
				Hyperoxia (Pw_{O_2} = 250 Torr; zero norepinephrine; N = 9)				
0	3·83 ± 0·10	3·41 ± 0·10	0·42 ± 0·10	192·3 ± 8·0‡	152·7 ± 8·1‡	0·48 ± 0·03‡	31·7 ± 1·5	20·5 ± 1·9
10	3·91 ± 0·05	3·40 ± 0·17	0·51 ± 0·12	190·7 ± 9·9‡	148·4 ± 10·1‡	0·48 ± 0·04‡	30·1 ± 1·7	19·4 ± 2·0
20	3·89 ± 0·06	3·34 ± 0·19	0·55 ± 0·11	177·7 ± 13·6‡	137·2 ± 14·3‡	0·44 ± 0·05‡	31·3 ± 2·2	20·3 ± 1·9

Values are means ± S.E.
* Significantly different from value at time zero ($P \leq 0·05$).
† Significantly different from value at time zero ($P \leq 0·01$).
‡ Significantly different from corresponding value in normoxic heads ($P \leq 0·001$).

Table 2. *The effect of perfusion flow rate ($\dot{Q}$) on oxygen transfer and haemodynamic variables in the isolated, saline-perfused head of rainbow trout*

Condition	Time (min)	$\dot{Q}$in (ml min^{-1})	$\dot{Q}$da (ml min^{-1})	Pa_{O_2} (Torr)	$Pa_{O_2}-Pv_{O_2}$ (Torr)	$\dot{M}_{O_2}$ (μmol min^{-1} 100 g^{-1})	Pin (cmH_2O)	Rbv (cmH_2O ml^{-1} min^{-1} 100 g^{-1})
'Normal' $\dot{Q}$	0	3·84 ± 0·06	3·34 ± 0·08	199·1 ± 6·3	160·0 ± 6·3	0·50 ± 0·06	39·0 ± 1·7	25·8 ± 2·2
Increased $\dot{Q}$	7·5	5·59 ± 0·10‡	4·81 ± 0·20‡	174·0 ± 6·0‡	131·8 ± 5·3‡	0·59 ± 0·05†	41·0 ± 2·5	18·7 ± 2·0‡
Decreased $\dot{Q}$	15	2·20 ± 0·10‡	1·91 ± 0·10‡	184·3 ± 9·6•	143·0 ± 6·1•	0·26 ± 0·04‡	29·2 ± 1·6‡	34·9 ± 5·3•
				Controls ($N = 6$)				
Control	0	4·00 ± 0·02	3·70 ± 0·05	203·7 ± 5·1	163·5 ± 5·3	0·52 ± 0·04	31·7 ± 1·5	20·5 ± 1·9
Control	7·5	4·01 ± 0·01	3·71 ± 0·02	196·0 ± 6·5	156·7 ± 6·0	0·49 ± 0·04	30·3 ± 1·7	19·6 ± 2·0
Control	15	4·02 ± 0·02	3·72 ± 0·02	189·3 ± 4·9§	149·0 ± 5·1§	0·47 ± 0·04§	30·6 ± 2·0	19·7 ± 1·9

Values are ± S.E., $N = 6$.
• Significantly different from value at normal $\dot{Q}$ ($P \leq 0{\cdot}05$).
† Significantly different from value at normal $\dot{Q}$ ($P \leq 0{\cdot}01$).
‡ Significantly different from value at normal $\dot{Q}$ ($P \leq 0{\cdot}001$).
§ Significantly different from value at time = zero in control group ($P \leq 0{\cdot}05$).

pressure to the branchial circulation was not increased significantly but Rbv was significantly decreased by 28%. No changes were observed in the perfusate flow distribution (in both cases, $\dot{Q}da$ represented approximately 87% of $\dot{Q}in$). Control preparations displayed no changes in any of the measured variables following 7·5 min perfusion (Table 2). Following 15 min perfusion, Qin was decreased to a level below 'normal' (2·2 ml min^{-1}). Although in this situation, Pa_{O_2} and $Pa_{O_2}-Pv_{O_2}$ were elevated to levels above those recorded at 7·5 min (increased flow), the overall changes were no different from those observed in controls after 15 min of perfusion. Thus, we conclude that reduced $\dot{Q}in$ did not affect Pa_{O_2} or $Pa_{O_2}-Pv_{O_2}$. Although Pin was significantly lower and Rbv higher than that recorded during either of the perfusion flow rates used, the perfusate flow distribution remained unchanged over this time period ($\dot{Q}da = 87\%$ of $\dot{Q}in$).

When the isolated head preparation was perfused for 20 min at 'normal' flow rate with physiological saline containing no epinephrine, there were progressive decreases in Pa_{O_2}, $Pa_{O_2}-Pv_{O_2}$, and as a consequence in $\dot{M}_{O_2}$ (Table 3). With the addition of 10^{-7} mol l^{-1} epinephrine, all measured variables remained stable throughout the experimental period, with the exception of Rbv, which decreased slightly (10%; Table 3). The addition of 10^{-6} mol l^{-1} epinephrine resulted in significant increases in

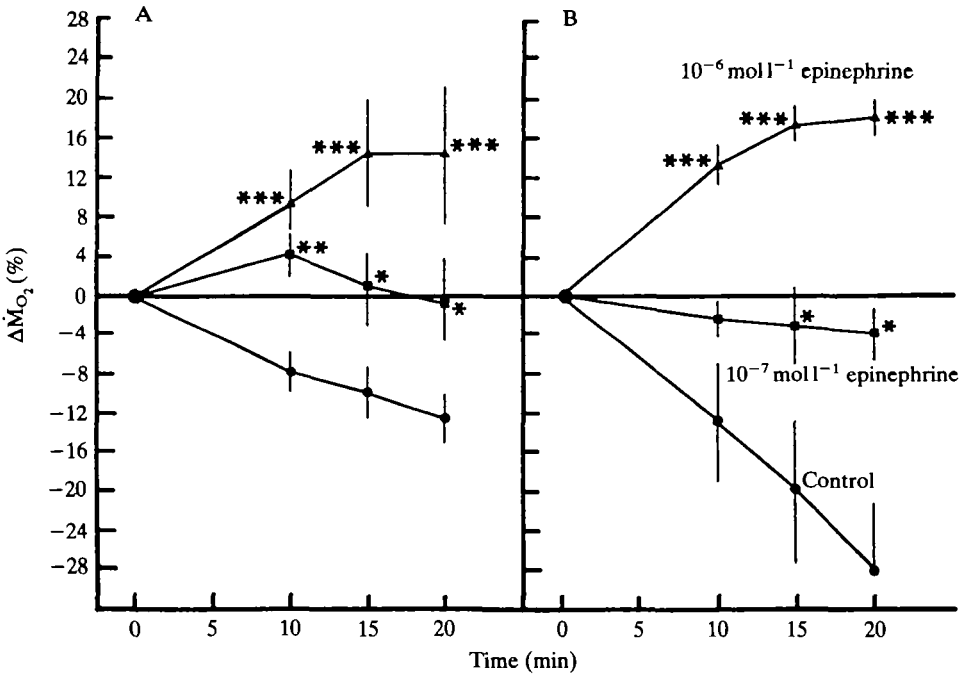

Fig. 2. The effect of epinephrine on $\dot{M}_{O_2}$, expressed as percentage change over time ($\Delta\dot{M}_{O_2}$), in the saline-perfused head of rainbow trout at (A) 'normal' perfusion flow rate (4 ml min^{-1}) and (B) at increased perfusion flow rate (6 ml min^{-1}). ●, Control ($N = 6$); ■, 10^{-7} mol l^{-1} epinephrine ($N = 6$); ▲, 10^{-6} mol l^{-1} epinephrine ($N = 6$). • Significantly different from control value ($P \leq 0{\cdot}05$). **($P \leq 0{\cdot}01$), ***($P \leq 0{\cdot}001$). Bars indicate ±1 S.E.

Table 3. *The effect of epinephrine on oxygen transfer and haemodynamic variables at 'normal' perfusion flow rate ($\dot{Q}$) in the isolated, saline-perfused trout head*

Time (min)	$\dot{Q}$in (ml min^{-1})	$\dot{Q}$da (ml min^{-1})	$\dot{Q}$av (ml min^{-1})	Pa_{O_2} (Torr)	$Pa_{O_2}-Pv_{O_2}$ (Torr)	$\dot{M}_{O_2}$ (μmol min^{-1} 100 g^{-1})	Pin (cmH_2O)	Rbv (cmH_2O ml^{-1} min^{-1} 100 g^{-1})
				Control ($N = 6$)				
0	4·00 ± 0·02	3·70 ± 0·05	0·29 ± 0·04	203·7 ± 5·1	163·5 ± 5·3	0·52 ± 0·04	31·7 ± 1·5	20·5 ± 1·9
10	4·01 ± 0·01	3·71 ± 0·02	0·30 ± 0·03	192·5 ± 6·7*	151·5 ± 6·2*	0·48 ± 0·04*	30·1 ± 1·7	19·4 ± 2·0
15	4·02 ± 0·02	3·72 ± 0·02	0·30 ± 0·03	189·3 ± 4·9*	149·0 ± 5·1*	0·47 ± 0·04*	30·6 ± 2·0	19·7 ± 1·9
20	4·00 ± 0·02	3·70 ± 0·03	0·30 ± 0·02	184·5 ± 7·6*	145·5 ± 8·6*	0·46 ± 0·05*	31·3 ± 2·2	20·3 ± 1·9
				10^{-7} mol l^{-1} epinephrine ($N = 6$)				
PE 0	3·87 ± 0·04	3·51 ± 0·08	0·36 ± 0·08	191·8 ± 3·5	145·2 ± 3·8	0·59 ± 0·03	36·4 ± 3·7	18·2 ± 1·5
10	3·87 ± 0·06	3·52 ± 0·08	0·35 ± 0·07	194·8 ± 4·7	152·5 ± 4·6	0·62 ± 0·05	33·3 ± 3·2	16·6 ± 1·4*
15	3·84 ± 0·04	3·46 ± 0·05	0·38 ± 0·06	189·8 ± 6·2	147·7 ± 6·3	0·60 ± 0·05	32·8 ± 3·3	16·5 ± 1·4*
20	3·85 ± 0·04	3·49 ± 0·07	0·36 ± 0·06	186·8 ± 7·5	144·3 ± 7·6	0·58 ± 0·05	32·6 ± 3·3	16·3 ± 1·4*
				10^{-6} mol l^{-1} epinephrine ($N = 6$)				
PE 0	3·80 ± 0·04	2·77 ± 0·20	1·04 ± 0·19	166·3 ± 5·7	128·5 ± 5·6	0·44 ± 0·05	39·5 ± 2·4	25·5 ± 2·1
10	3·84 ± 0·07	3·07 ± 0·18*	0·77 ± 0·19*	175·5 ± 4·6	138·2 ± 4·0	0·48 ± 0·05*	38·3 ± 3·3	23·7 ± 2·0*
15	3·86 ± 0·04	3·16 ± 0·16*	0·70 ± 0·17*	181·3 ± 4·8*	144·3 ± 3·0*	0·49 ± 0·04*	35·4 ± 3·2	22·2 ± 2·2*
20	3·85 ± 0·06	3·15 ± 0·14*	0·69 ± 0·18*	179·0 ± 5·3	144·2 ± 2·6*	0·49 ± 0·04*	34·6 ± 3·0*	21·7 ± 2·1†

Values are means ± S.E.
* Significantly different from value at time = zero ($P \leq 0·05$).
† Significantly different from value at time = zero ($P \leq 0·01$).
PE, pre-epinephrine.

Table 4. *The effect of epinephrine on oxygen transfer and haemodynamic variables at increased perfusion flow rate ($\dot{Q}$) in the isolated, saline-perfused trout head*

Time (min)	$\dot{Q}$in (ml min^{-1})	$\dot{Q}$da (ml min^{-1})	$\dot{Q}$av (ml min^{-1})	Pa_{O_2} (Torr)	$Pa_{O_2}-Pv_{O_2}$ (Torr)	$\dot{M}_{O_2}$ (μmol min^{-1} 100 g^{-1})	Pin (cmH_2O)	Rbv (cmH_2O ml^{-1} min^{-1} 100 g^{-1})
				Control ($R = 5$)				
0	5·77 ± 0·10	5·03 ± 0·21	0·74 ± 0·18	183·6 ± 10·6	146·2 ± 10·9	0·68 ± 0·05	21·8 ± 2·1	9·3 ± 1·0
10	5·81 ± 0·11	4·84 ± 0·23†	0·96 ± 0·23*	167·0 ± 16·8	129·8 ± 16·8	0·61 ± 0·08	21·2 ± 2·4	9·0 ± 0·9
15	5·87 ± 0·13	4·88 ± 0·23*	0·99 ± 0·19*	155·4 ± 17·2*	117·6 ± 16·1*	0·56 ± 0·08*	21·2 ± 2·2	8·9 ± 0·9
20	5·84 ± 0·11	4·77 ± 0·24†	1·07 ± 0·23*	144·0 ± 17·0*	106·2 ± 15·5*	0·50 ± 0·08†	21·2 ± 2·3	9·0 ± 0·9
				10^{-7} mol l^{-1} epinephrine ($N = 5$)				
PE 0	5·62 ± 0·13	4·94 ± 0·20	0·68 ± 0·19	184·2 ± 8·5	143·8 ± 8·0	0·73 ± 0·04	35·0 ± 3·3	13·8 ± 1·3
10	5·58 ± 0·12	4·89 ± 0·24	0·69 ± 0·22	183·2 ± 10·3	141·6 ± 9·9	0·71 ± 0·05	35·6 ± 2·9	14·1 ± 1·1
15	5·53 ± 0·10	4·83 ± 0·23	0·70 ± 0·21	183·6 ± 11·2	141·8 ± 10·7	0·71 ± 0·05	35·6 ± 3·4	14·3 ± 1·3
20	5·50 ± 0·12	4·97 ± 0·16	0·53 ± 0·17	180·8 ± 7·8	140·4 ± 7·4	0·70 ± 0·03	35·8 ± 3·7	14·3 ± 1·4
				10^{-6} mol l^{-1} epinephrine ($N = 6$)				
PE 0	5·82 ± 0·12	4·53 ± 0·25	1·29 ± 0·35	180·5 ± 5·7	140·2 ± 6·6	0·77 ± 0·09	22·6 ± 2·2	8·6 ± 0·9
10	5·92 ± 0·09	5·21 ± 0·19*	0·71 ± 0·16*	195·7 ± 7·7*	156·8 ± 7·8‡	0·88 ± 0·10‡	18·9 ± 2·0	7·1 ± 0·8*
15	5·93 ± 0·12	5·21 ± 0·18*	0·72 ± 0·16*	200·2 ± 6·5‡	161·5 ± 6·2‡	0·91 ± 0·10‡	18·7 ± 2·1*	7·0 ± 0·8*
20	5·91 ± 0·11	5·13 ± 0·20*	0·78 ± 0·17*	202·8 ± 5·6‡	163·0 ± 6·2‡	0·91 ± 0·10‡	18·6 ± 2·0*	7·0 ± 0·8*

Values are means ± S.E.
* Significantly different from pre-epinephrine value or value at time = zero ($P \leq 0·05$).
† Significantly different from pre-epinephrine value or value at time = zero ($P \leq 0·01$).
‡ Significantly different from pre-epinephrine value or value at time = zero ($P \leq 0·001$).
PE, pre-epinephrine.

Pa_{O_2}, $Pa_{O_2}-Pv_{O_2}$ and $\dot{M}_{O_2}$ which endured throughout the 20-min period. At 10 min, $\dot{Q}da$ was significantly elevated while $\dot{Q}av$ was significantly reduced. Pin and Rbv declined throughout the experiment, although Pin was significantly lower than the pre-epinephrine value only after 20 min.

Table 4 summarizes the results of identical experiments performed on preparations perfused at higher $\dot{Q}in$ (approximately 1·5×'normal'). In the absence of epinephrine, there were more pronounced decreases in Pa_{O_2}, $Pa_{O_2}-Pv_{O_2}$ and hence $\dot{M}_{O_2}$, with time. The proportional distribution of flow ($\dot{Q}da/\dot{Q}av$) decreased significantly during

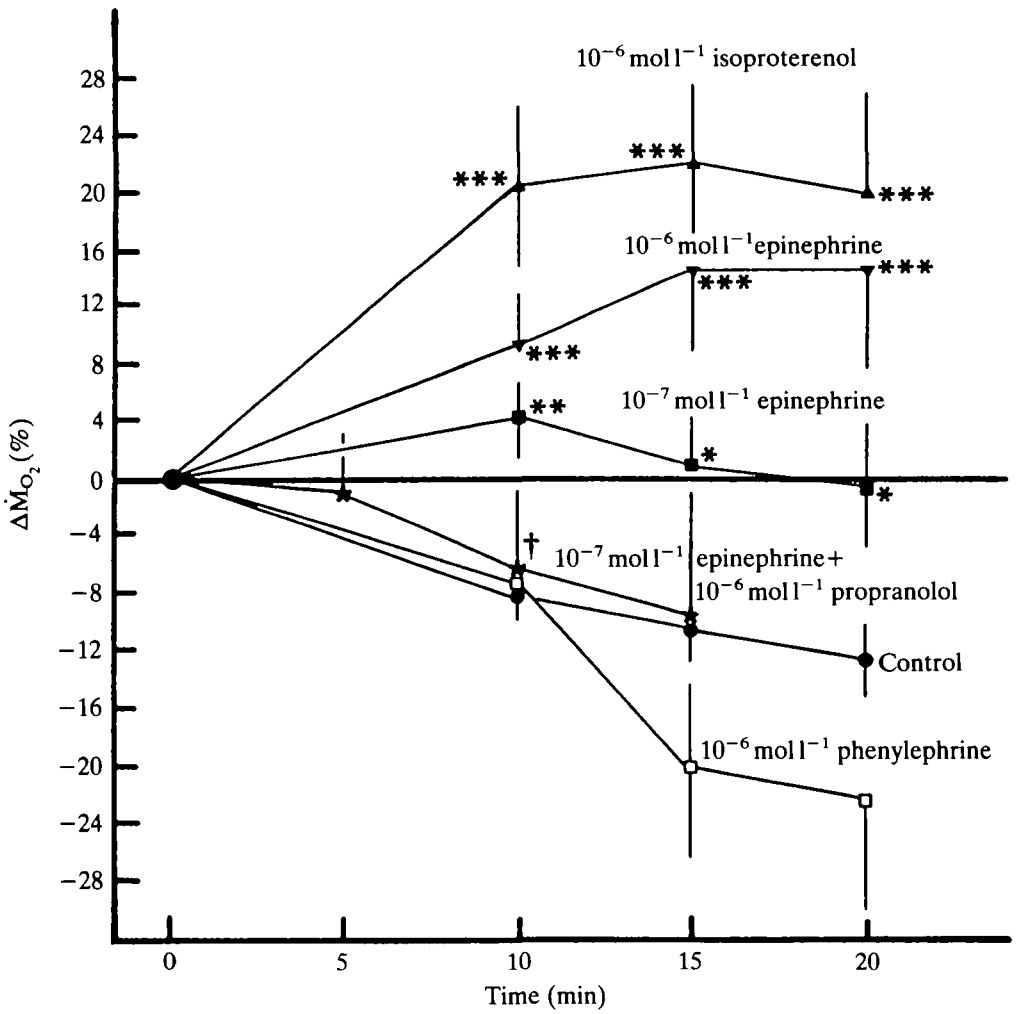

Fig. 3. The effect of epinephrine, adrenergic agonists and adrenergic antagonists on $\dot{M}_{O_2}$, expressed as percentage change over time ($\Delta\dot{M}_{O_2}$), in the saline-perfused head of rainbow trout at 'normal' $\dot{Q}in$. ▲, 10^{-6} mol l^{-1} isoprenaline ($N=8$); ▼, 10^{-6} mol l^{-1} epinephrine ($N=6$); ■, 10^{-7} mol l^{-1} epinephrine ($N=6$); ●, control ($N=6$); ★, 10^{-7} mol l^{-1} epinephrine + 10^{-6} mol l^{-1} propranolol ($N=5$); □, 10^{-6} mol l^{-1} phenylephrine ($N=4$). * Significantly different from control value ($P\leq 0{\cdot}05$), ** ($P\leq 0{\cdot}01$); †, significantly different from 10^{-7} mol l^{-1} epinephrine value. Bars indicate ± 1 S.E.

Table 5. *The effect of adrenergic stimulation on gill O_2 extraction effectiveness in the isolated, saline-perfused head of rainbow trout*

Time (min)	Control ($N = 6$)	10^{-7} mol l^{-1} epinephrine ($N = 6$)	O_2 extraction effectiveness (%)		
			10^{-6} mol l^{-1} epinephrine ($N = 6$)	10^{-6} mol l^{-1} isoproterenol ($N = 8$)	10^{-6} mol l^{-1} phenylephrine ($N = 4$)
Pre-treatment (0)	77·9 ± 2·4	68·1 ± 3·3	60·0 ± 2·5	64·2 ± 3·3	74·6 ± 2·9
10	72·7 ± 3·2†	70·2 ± 3·9	65·0 ± 1·9	74·8 ± 2·2†	68·1 ± 3·4
15	71·1 ± 2·4†	67·9 ± 3·7	67·9 ± 1·8*	75·3 ± 2·4†	59·5 ± 6·7*
20	68·9 ± 3·8†	66·3 ± 3·6	67·1 ± 1·9*	73·7 ± 2·7†	57·8 ± 7·5*
Overall change	−11·6 %	−2·6 %	+11·8 %	+14·8 %	−22·5 %

Values are means ± S.E.
* Significantly different from pre-treatment value ($P \leq 0{\cdot}05$).
† Significantly different from pre-treatment value ($P \leq 0{\cdot}01$).

Table 6. *The effect of adrenergic agents on oxygen transfer and haemodynamic variables in the isolated, saline-perfused trout head under normal perfusion flow conditions*

Time (min)	$\dot{Q}$in (ml min^{-1})	$\dot{Q}$da (ml min^{-1})	$\dot{Q}$av (ml min^{-1})	Pa_{O_2} (Torr)	$Pa_{O_2}-Pv_{O_2}$ (Torr)	$\dot{M}_{O_2}$ (μmol min^{-1} 100 g^{-1})	Pin (cmH_2O)	Rbv (cmH_2O ml^{-1} min^{-1} 100 g^{-1})
				10^{-6} mol l^{-1} isoprenaline ($N = 8$)				
PI 0	3·87 ± 0·09	2·83 ± 0·25	1·04 ± 0·26	178·9 ± 7·1	134·4 ± 6·6	0·43 ± 0·03	36·8 ± 2·0	23·3 ± 2·0
10	3·89 ± 0·09	3·01 ± 0·25†	0·88 ± 0·27*	200·4 ± 4·7‡	159·0 ± 4·8†	0·51 ± 0·03†	29·1 ± 2·1‡	18·3 ± 1·7‡
15	3·86 ± 0·09	3·01 ± 0·23†	0·85 ± 0·25†	201·4 ± 5·2†	160·8 ± 5·3†	0·51 ± 0·03†	27·8 ± 2·2‡	17·6 ± 1·6‡
20	3·90 ± 0·08	3·09 ± 0·21†	0·81 ± 0·23†	198·0 ± 5·8 †	157·1 ± 5·9†	0·50 ± 0·03†	27·9 ± 2·3‡	17·5 ± 1·6‡
				10^{-6} mol l^{-1} phenylephrine ($N = 4$)				
PØ 0	4·06 ± 0·04	2·95 ± 0·20	1·11 ± 0·22	196·5 ± 6·3	157·0 ± 5·7	0·54 ± 0·03	33·3 ± 5·4	19·3 ± 2·9
10	4·13 ± 0·07	3·03 ± 0·15	1·10 ± 0·17	183·0 ± 7·0*	143·0 ± 7·1*	0·50 ± 0·04	40·8 ± 7·2*	23·3 ± 4·1*
15	4·14 ± 0·04	3·05 ± 0·12	1·09 ± 0·15	164·8 ± 14·0*	125·0 ± 14·0*	0·43 ± 0·05*	43·0 ± 7·2*	24·3 ± 3·6*
20	4·10 ± 0·04	3·03 ± 0·14	1·07 ± 0·15	160·8 ± 15·5*	122·3 ± 16·0*	0·42 ± 0·06*	44·5 ± 7·8*	25·3 ± 0·9*
				10^{-7} mol l^{-1} epinephrine + 10^{-6} mol l^{-1} propranolol ($N = 5$)				
PP 0	4·05 ± 0·14	3·50 ± 0·13	0·55 ± 0·10	189·0 ± 6·3	145·8 ± 6·6	0·45 ± 0·03	33·6 ± 3·9	22·2 ± 1·9
5	3·98 ± 0·18	3·38 ± 0·16	0·60 ± 0·16	192·0 ± 10·3	149·2 ± 9·5	0·45 ± 0·03	36·0 ± 4·1	24·2 ± 2·1*
10	4·01 ± 0·16	3·29 ± 0·21	0·72 ± 0·19	182·6 ± 12·4	139·8 ± 11·5	0·42 ± 0·04	38·0 ± 4·6*	25·3 ± 2·1†
15	3·99 ± 0·18	3·27 ± 0·21	0·72 ± 0·18	177·0 ± 16·6	135·2 ± 15·2	0·41 ± 0·06	41·6 ± 4·6†	27·8 ± 2·2†

Values are means ± S.E.
* Significantly different from value at time = zero ($P \leq 0·05$).
† Significantly different from value at time = zero ($P \leq 0·01$).
‡ Significantly different from value at time = zero ($P \leq 0·001$).
PI, pre-isoprenaline; PØ, pre-phenylephrine; PP, pre-epinephrine + propanolol.

20 min while Pin and Rbv remained constant. Addition of 10^{-7} mol l^{-1} epinephrine under high flow conditions again had a stabilizing effect upon the measured variables over the experimental time course. Addition of 10^{-6} mol l^{-1} epinephrine resulted in marked increases in Pa_{O_2}, $Pa_{O_2}-Pv_{O_2}$, $\dot{Q}da$ and $\dot{M}_{O_2}$, while $\dot{Q}av$, Pin and Rbv were decreased significantly. Selected data from Tables 3 and 4 are presented graphically in Fig. 2.

Fig. 3 and Table 5 illustrate the effects of adrenergic agonists and antagonists on $\dot{M}_{O_2}$ and O_2 extraction effectiveness while perfusing at 'normal' flow rate. In terms of effectiveness in increasing O_2 uptake; 10^{-6} mol l^{-1} isoproterenol > 10^{-6} mol l^{-1} epinephrine > 10^{-7} mol l^{-1} epinephrine > control > 10^{-7} mol l^{-1} epinephrine + propanolol > 10^{-6} mol l^{-1} phenylephrine. Although deterioration of $\dot{M}_{O_2}$ and O_2 ext. eff., in the presence of phenylephrine, was greater than that observed in controls (Fig. 3; Table 5), these changes were not significantly different from controls.

As well as increasing Pa_{O_2}, $Pa_{O_2}-Pv_{O_2}$ and $\dot{M}_{O_2}$, the addition of 10^{-6} mol l^{-1} isoproterenol caused significant reductions in Pin and Rbv as well as an increase in $\dot{Q}da$ and a decrease in $\dot{Q}av$ (Table 6). In contrast, the addition of 10^{-6} mol l^{-1} phenylephrine to the perfusate resulted in significant decreases in Pa_{O_2}, $Pa_{O_2}-Pv_{O_2}$ and $\dot{M}_{O_2}$. Despite the fact that under this condition, Pin and Rbv were significantly increased, there was no change in perfusate flow distribution. Epinephrine, in the presence of propranolol, resulted in a less stable preparation with respect to Pa_{O_2}, $Pa_{O_2}-Pv_{O_2}$ and $\dot{M}_{O_2}$ compared to preparations perfused only with 10^{-7} mol l^{-1} epinephrine (Table 3) and in this respect closely resembled the results of control perfusions (Table 3). The increases in Pin and Rbv during perfusion with epinephrine plus propranolol reflect the alpha-constrictory component of epinephrine.

The data in Tables 3–6 are somewhat complicated because of occasional significant differences between the various groups in their starting levels of Pa_{O_2}, $\dot{M}_{O_2}$, Pin and Rbv. However, due to the paired design of the experiments (i.e. each fish acting as its own control) we are confident that these differences have not affected the interpretation of the results.

DISCUSSION

Irrigation of the gills with moderately hyperoxic water ($Pw_{O_2} \simeq 250$ Torr) allowed us to achieve a stable, short-term (20 min), saline-perfused head preparation in the absence of epinephrine. It is important to note that external hyperoxia did not cause an increase in branchial vascular resistance to flow, and we therefore believe that hyperoxic vasoconstriction was not occurring in the perfused head preparation. In contrast, Wood & Jackson (1980) have suggested that exposure of trout to hyperoxic water results in a decrease in functional surface area of the gill, presumably as a result of branchial vasoconstriction. A possible explanation for the discrepancy is the much higher external P_{O_2} (350–650 Torr) employed by Wood & Jackson (1980) compared with the present study (250 Torr). The stabilizing effect of hyperoxic water suggests that the progressive increase in Rbv and decrease in Pa_{O_2} during normoxia is due to

tissue hypoxaemia and may reflect the low O_2-carrying capacity of the perfusate. Clearly, irrigation of the gills with hyperoxic water does not simulate *in vivo* conditions yet we have used this technique to obviate the need for excessive levels of catecholamines in the perfusate in order to achieve a stable preparation. We believe the conditions for O_2 diffusion to be comparable in hyperoxic *versus* normoxic heads as indicated by similar values for $\dot{Q}$da, $\dot{Q}$av, Pin, Rbv and O_2 extraction effectiveness ($\simeq$10 % greater in hyperoxic preparations). Using this technique, we were able to demonstrate that increasing $\dot{Q}$in causes a reduction of Pa_{O_2}. This result indicates that perfusate transit time through the gill vasculature is an important factor determining oxygen extraction efficiency and that, clearly, there are diffusion limitations to oxygen transfer in the perfused head preparation, over the physiological range of $\dot{Q}$ values employed. The inhibitory effect of increased $\dot{Q}$in on Pa_{O_2} was evident even though gill surface area was probably increased, and percentage utilization of saline O_2 by metabolism at post-lamellar sites (Daxboeck *et al.* 1982) was probably reduced during elevated $\dot{Q}$in. The decrease of Rbv at high $\dot{Q}$in reflects the compliant nature of the gill vasculature (Farrell, Daxboeck & Randall, 1979). The lack of an effect of reduced $\dot{Q}$in on Pa_{O_2} may be due to greater percentage utilization of saline O_2 by metabolizing tissue (transit time limitation), thereby obscuring any increase in gill O_2 diffusion. Alternatively, O_2 diffusion limitations may be absent at flow rates below normal. It is difficult to extrapolate results from perfused preparations to the intact animal, especially when saline is used as the perfusion medium. Problems associated with using saline as a perfusate include its low O_2-carrying capacity and, as a result, low $\dot{M}_{O_2}$, a linear O_2 dissociation curve and the possibility of severe and irreversible lamellar oedema (Ellis & Smith, 1983). However, results of a recent study (Perry, Lauren & Booth, 1984) have shown lack of oedema or other structural abnormalities in gills of saline-perfused trout heads. This finding, together with the stable Rbv observed in the present study, indicates that no additional structural barriers to O_2 diffusion are forming during the course of experiments. Other limitations of the perfused head are artificial and sometimes elevated gill ventilation (approximately five times the normal *in vivo* value in the present investigation) and absence of Pda which probably affect, respectively, the P_{O_2} gradient profile across the gill and the pattern of flow through the branchial vasculature. Nevertheless, we believe the results of this study may be representative of the situation in intact fish because, as in *in vivo* experiments (Holeton & Randall, 1967; Cameron & Davis, 1970; Randall, 1970), Pa_{O_2} was approximately 75 % of Pw_{O_2} under control conditions (zero catecholamine content, 'normal' $\dot{Q}$). Moreover, the use of a low O_2 capacitance perfusate serves to decrease diffusion limitations to gill O_2 transfer, and therefore one might predict a greater effect of increased $\dot{Q}$ on Pa_{O_2} if blood was perfusing the gills. Results of previous perfusion studies (Daxboeck *et al.* 1982; P. Pärt, personal communication) have shown Pa_{O_2} to be insensitive to flow adjustments and as such disagree with the results of this study. The lack of diffusion limitations in the previous studies was probably due to the high level of catecholamines present. P. Pärt (personal communication) used 10^{-6} mol l^{-1} epinephrine in the perfusate while the preparation of Daxboeck *et al.* (1982) may have contained high levels of circulating

catecholamines due to the stressed condition of the animal. Catecholamines are known to enhance gill oxygen diffusing capacity (Wood, McMahon & McDonald, 1978; Pettersson & Johansen, 1982; Pärt *et al.* 1982; Pettersson, 1983), so it is not surprising that O_2 transfer was perfusion limited and therefore insensitive to adjustments of $\dot{Q}$in in these experiments.

Knowing that increased flow rate reduces gill O_2 extraction efficiency or imposes additional diffusion limitations, one might predict that epinephrine would have a greater effect on stimulating O_2 transfer at higher perfusion flow rates. Indeed, this is the exact result observed in the present study (Fig. 2). This phenomenon may be of physiological significance during exercise, when cardiac output is increased (see Jones & Randall, 1978). Thus, epinephrine released into the blood may enable the exercising fish to maintain O_2 transfer and arterial P_{O_2} constant (Stevens & Randall, 1967; Kiceniuk & Jones, 1977), even though transit time through the gill is reduced. Clearly, possible diffusion limitations imposed by increased cardiac output are offset by the effect of epinephrine on increasing gill O_2 diffusing capacity. The levels of epinephrine used in the present study (10^{-6}–10^{-7} mol l^{-1}) are similar to levels measured in stressed fish (Nakano & Tomlinson, 1967; Mazeaud, Mazeaud & Donaldson, 1977) and resemble levels in exercising fish (D. J. Randall, personal communication).

As well as enhancing branchial O_2 uptake, epinephrine is known to affect gill haemodynamics (Keys & Bateman, 1932; Ostlund & Fange, 1962; Richards & Fromm, 1969; Bergman, Olson & Fromm, 1974; Wood, 1975; Girard & Payan, 1976; Payan & Girard, 1977; Booth, 1979; Claiborne & Evans, 1980; Pettersson, 1983), water flux (Isaia, Payan & Girard, 1979) and ionic transport (see Girard & Payan, 1980). The haemodynamic effects of epinephrine are bimodal, primarily composed of an alpha constrictory component acting at the level of the arterio-venous anastomoses (AVAs) and a beta dilatory component acting at the level of the afferent lamellar arterioles (ALAs; see review by Laurent, 1982). The net result is a decrease in branchial vascular resistance and an increase in $\dot{Q}$da (Tables 3, 4). More importantly, the haemodynamic effects of epinephrine cause a greater surface area of the gill to be perfused *via* lamellar recruitment (Holbert, Boland & Olson, 1979; Booth, 1979). Thus, the observed effects of epinephrine on oxygen, ion and water transport may be a result of lamellar recruitment. An alternative explanation is that epinephrine increases the permeability of the gill epithelium to small lipophilic and water-soluble substances as proposed by Haywood, Isaia & Maetz (1977). In an attempt to differentiate between these two possibilities, we examined the effects of adrenergic agonists and antagonists on oxygen uptake. Unlike the results of Pettersson (1983), which demonstrated stimulation of $\dot{M}_{O_2}$ following both alpha- and beta-adrenergic stimulation (primarily alpha) in the cod head, the results of the present study show that, in the trout head, $\dot{M}_{O_2}$ is enhanced only by beta-receptor stimulation whereas alpha-receptor stimulation (both by addition of phenylephrine and beta blockade) had no significant effect on $\dot{M}_{O_2}$. Beta-receptor stimulation is believed to cause dilation of ALAs (Girard & Payan, 1976; Claiborne & Evans, 1980), which is consistent with lamellar recruitment. However, beta-adrenergic stimulation also increases gill

epithelial permeability to water and perhaps oxygen (see review by Isaia, 1984). Thus, the observed stimulation of $\dot{M}_{O_2}$ following beta-adrenergic stimulation could be due to either of these two possibilities. Alpha-receptor stimulation is believed to cause constriction of AVAs (Claiborne & Evans, 1980). In our study, addition of phenylephrine resulted in an increase in Pin and Rbv with no change in $\dot{Q}$da or $\dot{Q}$av. These results are not consistent with constriction of AVAs but imply a site of action proximal to the AVAs. Pettersson (1983) has suggested the possibility of alpha-adrenergic constriction of efferent lamellar arterioles (ELAs) to explain this result. Constriction of ELAs would lead to lamellar recruitment (Pettersson, 1983) and result in increased $\dot{M}_{O_2}$, but this was not observed in the present study (Fig. 3). The lack of an effect of phenylephrine on $\dot{M}_{O_2}$ might be a result of reduced gill epithelial permeability to oxygen. Thus, even if lamellar recruitment were occurring following alpha-adrenergic stimulation, clearly the decrease in epithelial permeability must negate this effect so that the net result is no change in $\dot{M}_{O_2}$. That this result was not observed by Pettersson (1983) may reflect species differences or the high diffusion limitations in his preparation, as suggested by low O_2 extraction effectiveness (23 % without epinephrine, 43 % with 10^{-6} mol l^{-1} epinephrine). In other words, the stimulatory effect of lamellar recruitment on $\dot{M}_{O_2}$ may have predominated since O_2 uptake was already severely limited.

The overall effect of epinephrine addition is an increase in gill oxygen uptake and is the result of both alpha- and beta-receptor stimulation. This effect can be accounted for by beta-adrenergic increase in surface area and/or increase in gill permeability plus alpha-adrenergic increase in surface area (if it occurs) minus alpha-adrenergic decrease in gill permeability (if it occurs). Clearly, the beta effects must predominate to explain the net result. This is similar to the argument which is used to explain the decrease in gill resistance following epinephrine application, in which the overall result is the sum of opposing alpha and beta effects, with the beta dilatory effects predominating (Payan & Girard, 1977).

In summary, the results presented above demonstrate that in the saline-perfused trout head preparation, and perhaps in the intact animal, there are diffusion limitations to branchial O_2 transfer which increase with increasing $\dot{Q}$. Epinephrine enhances $\dot{M}_{O_2}$ by increasing the number of lamellae perfused (increase in gill surface area) and/or by increasing gill epithelial permeability to oxygen. These effects are mediated primarily by beta-receptors. Alpha-receptor stimulation did not affect $\dot{M}_{O_2}$ although our results suggest alpha-receptor-mediated constriction of ELAs (increased Rbv, no effect on $\dot{Q}$da/$\dot{Q}$av) which would cause lamellar recruitment. Thus, we speculate that alpha-adrenergic stimulation may decrease gill permeability to oxygen. Presumably, during epinephrine treatment the beta effects dominate so that the overall result is an increase in gill oxygen uptake.

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REFERENCES

BERGMAN, H. L., OLSON, K. & FROMM, P. O. (1974). The effects of vaso-active agents on the functional surface area of the isolated perfused gills of rainbow trout. *J. comp. Physiol.* **94**, 267–286.

BOOTH, J. H. (1979). The effects of oxygen supply, epinephrine and acetylcholine on the distribution of blood flow in trout gills. *J. exp. Biol.* **83**, 31–39.

CAMERON, J. N. & DAVIS, J. C. (1970). Gas exchange in rainbow trout with varying blood oxygen capacity. *J. Fish Res. Bd Can.* **27**, 1069–1085.

CLAIBORNE, J. B. & EVANS, D. H. (1980). The isolated perfused head of the teleost fish, *Myoxocephalus octodecimspinosus*: hemodynamic effects of epinephrine. *J. comp. Physiol.* **138**, 79–85.

DAVIE, P. S. & DAXBOECK, C. (1983). Modification of a piston-type perfusion pump for delivery of low flow rates. *Experientia* **39**, 433–434.

DAXBOECK, C., DAVIE, P. S., PERRY, S. F. & RANDALL, D. J. (1982). Oxygen uptake in a spontaneously ventilating, blood-perfused trout preparation. *J. exp. Biol.* **101**, 35–45.

ELLIS, A. G. & SMITH, D. G. (1983). Edema formation and impairment of O_2 transfer in Ringer-perfused gills of the eel, *Anguilla australis*. *J. exp. Zool.* **227**, 371–380.

FARRELL, A. P., DAXBOECK, C. & RANDALL, D. J. (1979). The effect of input pressure and flow on the pattern and resistance to flow in the isolated perfused gill of a teleost fish. *J. comp. Physiol.* **133**, 233–240.

FISHER, T., COBURN, R. & FORSTER, R. (1969). Carbon monoxide diffusing capacity in the bullhead catfish. *J. appl. Physiol.* **26**, 161–169.

GIRARD, J. P. & PAYAN, P. (1976). Effect of epinephrine on vascular space of gills and head of rainbow trout. *Am. J. Physiol.* **230**, 1555–1560.

GIRARD, J. P. & PAYAN, P. (1980). Ion exchange through respiratory and chloride cells in freshwater and seawater adapted teleosteans. *Am. J. Physiol.* **238**, 260–268.

HAYWOOD, G. P., ISAIA, J. & MAETZ, J. (1977). Epinephrine effects on branchial water and urea flux in rainbow trout. *Am. J. Physiol.* **232**, 110–115.

HOLBERT, P. W., BOLAND, E. J. & OLSON, K. R. (1979). The effect of epinephrine and acetylcholine on the distribution of red cells within the gills of the channel catfish (*Ictalurus punctatus*). *J. exp. Biol.* **79**, 135–146.

HOLETON, G. F. & RANDALL, D. J. (1967). The effect of hypoxia upon the partial pressures of gases in blood and water afferent and efferent to the gills of rainbow trout. *J. exp. Biol.* **46**, 317–327.

ISAIA, J. (1984). Water and non-electrolyte permeation. In *Fish Physiology*, Vol. XB, (eds W. S. Hoar & D. J. Randall), pp. 1–38. New York: Academic Press.

ISAIA, J., PAYAN, P. & GIRARD, J. P. (1979). A study of the water permeability of the gills of freshwater- and seawater-adapted trout (*Salmo gairdneri*): mode of action of epinephrine. *Physiol. Zoöl.* **52**, 269–279.

JONES, D. R. & RANDALL, D. J. (1978). The respiratory and circulatory systems during exercise. In *Fish Physiology*, Vol. VII, (eds W. S. Hoar & D. J. Randall), pp. 425–501. New York: Academic Press.

KEYS, A. & BATEMAN, J. B. (1932). Branchial responses to adrenaline and pitressin in the eel. *Biol. Bull. mar. Biol. Lab., Woods Hole* **63**, 327–336.

KICENIUK, J. W. & JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.

LAURENT, P. (1982). Structure of vertebrate gills. In *Gills*, Society for Experimental Biology Seminar Series 16, (eds D. F. Houlihan, J. C. Rankin & T. J. Shuttleworth), pp. 25–43. New York: Cambridge University Press.

MAZEAUD, M. M., MAZEAUD, F. & DONALDSON, E. M. (1977). Primary and secondary effects of stress in fish: some new data with a general review. *Trans. Am. Fish. Soc.* **106**, 201–211.

NAKANO, T. & TOMLINSON, N. (1967). Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. *J. Fish. Res. Bd Can.* **24**, 1701–1714.

ODULEYE, S. O., CLAIBORNE, J. B. & EVANS, D. H. (1982). The isolated, perfused head of the toadfish, *Opsanus beta*. I. Vasoactive responses to cholinergic and adrenergic stimulation. *J. comp. Physiol.* **149**, 107–113.

OSTLUND, E. & FANGE, R. (1962). Vasodilation by adrenaline and noradrenaline, and the effects of some other substances on perfused fish gills. *Comp. Biochem. Physiol.* **5**, 307–309.

PÄRT, P., TUURALA, H. & SOIVIO, A. (1982). Oxygen transfer, gill resistance and structural changes in rainbow trout (*Salmo gairdneri*) gills perfused with vasoactive agents. *Comp. Biochem. Physiol.* **71C**, 7–13.

PAYAN, P. & GIRARD, J. P. (1977). Adrenergic receptors regulating patterns of blood flow through the gills of trout. *Am. J. Physiol.* **232**, 18–23.

PAYAN, P. & MATTY, A. J. (1975). The characteristics of ammonia excretion by a perfused isolated head of trout (*Salmo gairdneri*): effect of temperature and CO_2-free Ringer. *J. comp. Physiol.* **96**, 167–184.

PERRY, S. F., HASWELL, M. S., RANDALL, D. J. & FARRELL, A. P. (1981). Branchial ionic uptake and acid-base regulation in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **92**, 289–303.

PERRY, S. F., LAUREN, D. J. & BOOTH, C. E. (1984). Absence of branchial edema in perfused heads of rainbow trout (*Salmo gairdneri*). *J. exp. Zool.* **231**, 441–445.

PERRY, S. F., PAYAN, P. & GIRARD, J. P. (1984). Adrenergic control of branchial chloride transport in the isolated, saline-perfused head of the freshwater trout (*Salmo gairdneri*). *J. comp. Physiol.* **154**, 269–274.

PETTERSSON, K. (1983). Adrenergic control of oxygen transfer in perfused gills of the cod, *Gadus morhua*. *J. exp. Biol.* **102**, 327–335.

PETTERSSON, K. & JOHANSEN, K. (1982). Hypoxic vasoconstriction and the effects of adrenaline on gas exchange efficiency in fish gills. *J. exp. Biol.* **97**, 263–272.

PEYRAUD-WAITZENEGGER, M. (1979). Simultaneous modifications of ventilation and arterial P_{O_2} by catecholamines in the eel, *Anguilla anguilla*: participation of alpha and beta effects. *J. comp. Physiol.* **129**, 343–354.

RANDALL, D. J. (1970). The circulatory system. In *Fish Physiology*, Vol. IV, (eds W. S. Hoar & D. J. Randall), p. 133. New York: Academic Press.

RICHARDS, B. D. & FROMM, P. O. (1969). Patterns of blood flow through filaments and lamellae of isolated perfused trout gills. *Am. Zool.* **8**, 766–771.

STEEN, J. B. & KRUYSSE, A. (1964). The respiratory function of teleostean gills. *Comp. Biochem. Physiol.* **12**, 127–142.

STEVENS, E. D. & RANDALL, D. J. (1967). Changes in gas concentrations in blood and water during moderate swimming activity in rainbow trout. *J. exp. Biol.* **46**, 329–337.

WOOD, C. M. (1975). A pharmacological analysis of the adrenergic and cholinergic mechanisms regulating branchial vascular resistance in the rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* **53**, 1569–1577.

WOOD, C. M. & JACKSON, E. B. (1980). Blood acid-base regulation during environmental hyperoxia in the rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **42**, 351–372.

WOOD, C. M., MCMAHON, B. R. & MCDONALD, D. G. (1978). Oxygen exchange and vascular resistance in the totally perfused rainbow trout. *Am. J. Physiol.* **234**, 201–208.

WOOD, C. M., MCMAHON, B. R. & MCDONALD, D. G. (1979). Respiratory, ventilatory and cardiovascular responses to experimental anaemia in the starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **82**, 139–162.

ZAR, J. H. (1974). *Biostatistical Analysis*. Prentice-Hall Biological Sciences Series (eds W. D. McElroy & C. P. Swanson). New Jersey: Prentice-Hall, Inc.