J Comp Physiol B (2001) 171: 623–634
DOI 10.1007/s003600100213

ORIGINAL PAPER

S.F. Perry · C.J. Montpetit · J. McKendry
P.R. Desforges · K.M. Gilmour · C.M. Wood
K.R. Olson

The effects of endothelin-1 on the cardiorespiratory physiology of the freshwater trout (*Oncorhynchus mykiss*) and the marine dogfish (*Squalus acanthias*)

Accepted: 28 May 2001 / Published online: 9 August 2001

Abstract The aim of the present study was to evaluate the effects of endothelin-1-elicited cardiovascular events on respiratory gas transfer in the freshwater rainbow trout (*Oncorhynchus mykiss*) and the marine dogfish (*Squalus acanthias*). In both species, endothelin-1 (666 pmol kg^{-1}) caused a rapid (within 4 min) reduction (ca. 30–50 mmHg) in arterial blood partial pressure of O_2. The effects of endothelin-1 on arterial blood partial pressure of CO_2 were not synchronised with the changes in O_2 partial pressure and the responses were markedly different in trout and dogfish. In trout, arterial CO_2 partial pressure was increased transiently by ~1.0 mmHg but the onset of the response was delayed and occurred 12 min after endothelin-1 injection. In contrast, CO_2 partial pressure remained more-or-less constant in dogfish after injection of endothelin-1 and was increased only slightly (~0.1 mmHg) after 60 min. Pre-treatment of trout with bovine carbonic anhydrase (5 mg ml^{-1}) eliminated the increase in CO_2 partial pressure that was normally observed after endothelin-1 injection. In both species, endothelin-1 injection caused a decrease in arterial blood pH that mirrored the changes in CO_2 partial pressure. Endothelin-1 injection was associated with transient (trout) or persistent (dogfish) hyperventilation as indicated by pronounced increases in breathing frequency and amplitude. In trout, arterial blood pressure remained constant or was decreased slightly and was accompanied by a transient increase in systemic resistance, and a temporary reduction in cardiac output. The decrease in cardiac output was caused solely by a reduction in cardiac frequency; cardiac stroke volume was unaffected. In dogfish, arterial blood pressure was lowered by ~10 mmHg at 6–10 min after endothelin-1 injection but then was rapidly restored to pre-injection levels. The decrease in arterial blood pressure reflected an increase in branchial vascular resistance (as determined using in situ perfused gill preparations) that was accompanied by simultaneous decreases in systemic resistance and cardiac output. Cardiac frequency and stroke volume were reduced by endothelin-1 injection and thus both variables contributed to the changes in cardiac output. We conclude that the net consequences of endothelin-1 on arterial blood gases result from the opposing effects of reduced gill functional surface area (caused by vasoconstriction) and an increase in blood residence time within the gill (caused by decreased cardiac output.

Communicated by L.C.-H. Wang

S.F. Perry (✉) · C.J. Montpetit · J. McKendry · P.R. Desforges
Department of Biology, University of Ottawa,
30 Marie Curie, Ottawa, Ontario, Canada K1N 6N5
E-mail: sfperry@science.uottawa.ca
Tel.: +1 613-5625800 ext 6005
Fax: +1-613-5625486

K.M. Gilmour
Department of Biology, Carleton University,
1125 Colonel By Drive, Ottawa,
Ontario, Canada K1S 5B6

C.M. Wood
Department of Biology, McMaster University,
1280 Main Street W., Hamilton, Ontario, Canada L8S 4K1

K.R. Olson
Indiana University School of Medicine,
South Bend Center, University of Notre Dame,
Notre Dame, IN 46556-5607, USA

Keywords Catecholamines · Cardiac output · Ventilation · Endothelin-1 · Carbonic anhydrase

Abbreviations *CA* carbonic anhydrase · *ET-1* endothelin-1 · f_H cardiac frequency · f_R ventilation (respiration) frequency · G_{DIFF} diffusion conductance · H_{SV} cardiac stroke volume · P_a arterial blood pressure · P_{CA} caudal artery pressure · P_{DA} dorsal aortic pressure · $PaCO_2$ · arterial blood · PCO_2 · PaO_2 arterial PO_2 · *pHa* arterial pH · $\dot{Q}$ cardiac output · R_G branchial vascular resistance · R_S systemic vascular resistance · *Vent. Amp.* ventilation amplitude

Introduction

Endothelin-1 (ET-1) is a 21-amino acid peptide hormone that is produced predominantly, though not exclusively, in vascular endothelial cells (Miyauchi and Masaki 1999). In mammals (Masaki 1993) and lower vertebrates (Poder et al. 1991), ET-1 is a potent vasoconstrictor of systemic blood vessels via its interaction with ET_A or ET_B receptors (Neylon 1999). Typically, stimulation of vascular smooth muscle $ET_{A/B}$ receptors promotes direct vasoconstriction owing to accumulation of intracellular Ca^{2+} (Miyauchi and Masaki 1999). Stimulation of endothelial cell ET_B receptors, however, indirectly evokes vasodilation as a consequence of localised release of endothelium-derived relaxing factors (EDRFs), in particular nitric oxide (de Nucci et al. 1988). Indeed, it is the activation of ET_B receptors that promotes the pronounced vasodilatory response of the pulmonary vasculature to ET-1 in mammals (Muramatsu et al. 1999). In fish, the functional equivalent of the pulmonary vascular bed is the microcirculation of the gill. In marked contrast to the mammalian lung, however, ET-1 elicits pronounced vasoconstriction of the branchial circulation (Olson et al. 1991; Sundin and Nilsson 1998; Stenslokken et al. 1999; Hoagland et al. 2000). The blood channels within the gill lamella lack true endothelial cells and thus unlike in the pulmonary circulation, EDRFs cannot contribute to ET-1 induced vasodilatation. The mechanism underlying the branchial vasoconstriction is contraction of lamellar pillar cells (Stenslokken et al. 1999). The resultant narrowing of blood channels leads to a redistribution of lamellar blood flow whereby the peripheral or marginal channels are perfused preferentially at high velocity while the central channels comprising the lamellar vascular sheet receive reduced flow at low velocity (Sundin and Nilsson 1998; Stenslokken et al. 1999). The marginal channels (in particular the inner or basal channels) are less efficient sites of gas transfer (Farrell et al. 1980) and their efficiency would be lowered further by the increased velocity of flow (Stenslokken et al. 1999). Therefore, re-routing of blood away from the central vascular sheet and the resultant loss of functional surface area would be expected to lower gas transfer efficiency and cause a lowering of arterial PO_2 (PaO_2) and an increase of arterial PCO_2 ($PaCO_2$). The situation is somewhat more complex, however, because the branchial vasoconstriction associated with ET-1 injection is accompanied by a profound decrease in cardiac output ($\dot{Q}$; Stenslokken et al. 1999; Hoagland et al. 2000). Consequently, the transit or residence time of the remaining blood flowing within the central lamellar channels (Stenslokken et al. 1999) is increased. An increased transit time would extend the period for gas equilibration across the gill epithelium and this potentially could increase gas transfer efficiency (i.e. raise PaO_2 and lower $PaCO_2$). Thus, a decrease in $\dot{Q}$ could counteract the effects of reduced functional surface area on arterial blood gases (Stenslokken et al. 1999).

Therefore, the goal of this study was to assess the impact of these conflicting effects on arterial blood gas tensions. This was accomplished by continuous measurements of PaO_2, $PaCO_2$ and arterial pH (pHa) before and after intravascular injections of ET-1 in rainbow trout (*Oncorhynchus mykiss*) or Pacific spiny dogfish (*Squalus acanthias*). These two species were selected for comparison because of their presumed different strategies for CO_2 excretion. The trout relies exclusively on red blood cell (RBC) carbonic anhydrase (CA) to dehydrate plasma HCO_3^-, whereas the dogfish is thought to utilise both extracellular and RBC CA in branchial CO_2 transfer (Gilmour 1997; Tufts and Perry 1998; Gilmour et al. 2001). Thus, it was reasoned that the presence of extracellular CA in dogfish would limit the impact on CO_2 excretion of diffusion-limitations and transit time changes imposed by ET-1. Moreover, it was hypothesised that the addition of bovine CA to trout plasma in vivo would enable rapid extracellular dehydration of HCO_3^- and thus abolish the inter-specific differences in the $PaCO_2$ response to ET-1.

Materials and methods

Experimental animals

Pacific spiny dogfish (*S. acanthias*) were collected by net during trawls by local fishermen or angled by hook and line and transported to holding facilities at Bamfield Marine Station (BMS; Bamfield, Vancouver Island, British Columbia). The dogfish were kept under natural photoperiod in a 75,000-l opaque circular tank provided with aerated full-strength seawater (30–32 mg ml^{-1}) at 12°C and they were fed twice weekly with herring. In the present study a total of 18 dogfish (average mass = 1489 ± 131 g) were used within 4 weeks of their capture.

Rainbow trout (*O. mykiss*) of both sexes were obtained from Linwood Acres Trout Farm. All fish were kept in large fibreglass tanks supplied with flowing, aerated and dechlorinated, city of Ottawa tap water: $[Na^+] = 0.15$ mmol l^{-1}; $[Cl^-] = 0.15$ mmol l^{-1}; $[K^+] = 0.02$ mmol l^{-1}; $[Ca^{2+}] = 0.40$ mmol l^{-1}; $[HCO_3^-] = 0.41$ mmol l^{-1}; pH = 7.5–8.0; temperature = 13°C) under a constant 12 h light-12 h dark photoperiod. Trout were fed to satiation on alternate days with commercial trout pellets until 24 h prior to experimentation. All fish were allowed at least 2 weeks to acclimate to the holding conditions before any experiments were performed. Two groups of fish were used; for experiments involving the recording of cardiovascular and blood respiratory variables, fish ($n = 40$) weighing between 500 g and 800 g were used (average mass = 634 ± 21 g). Smaller fish were used for experiments designed to examine the effects of ET-1 on plasma catecholamine levels (average mass = 287 ± 12 g; $n = 16$).

Surgical procedures

In vivo experiments

Dogfish were immersed in an aerated anaesthetic solution of benzocaine (ethyl-*p*-aminobenzoate; 0.1 g l^{-1}) and transferred to an operating table where the gills were irrigated continuously with the same anaesthetic solution. Bi-directional cannulation of the coeliac

artery was required to establish an extracorporeal arterial blood circulation (see below). After making a ventral incision and externalising much of the viscera, the coeliac artery was cannulated in the orthograde and retrograde directions using polyethylene tubing (Clay-Adams PE 50). The viscera were re-inserted, the wound sutured, and the cannulae were secured firmly to the ventral musculature. A lateral incision was made in the caudal peduncle to expose and cannulate (PE 50; Clay-Adams) both the caudal vein and the caudal artery in the anterograde direction (Axelsson and Fritsche 1994). While the arterial cannula allowed caudal artery blood pressure (P_{CA}) measurements, the caudal vein cannula permitted injections and/or repeated blood sampling. All cannulae were filled with heparinised (100 IU ml^{-1} ammonium heparin; Sigma Chemical, St. Louis, Mo.) dogfish saline (500 mmol l^{-1} NaCl). In addition, the pericardial cavity was exposed with a ventral midline incision and the pericardium was dissected to expose the conus arteriosus. To enable measurement of cardiac output ($\dot{Q}$), a 3S or 4S ultrasonic flow probe (Transonic Systems, Ithaca, N.Y.) was placed non-occlusively around the conus. Lubricating jelly was used with the perivascular flowprobe as an acoustic couplant. Silk sutures were used to close the ventral and caudal peduncle incisions, and to anchor the cardiac output probe lead and the cannulae to the skin. In order to assess ventilatory amplitude and frequency, catheters (Clay-Adams PE 160) were inserted into the spiracular cavities and sutured to the head. After surgery, dogfish were placed into individual flow-through opaque acrylic or wooden boxes and left to recover for approximately 24 h before experimentation.

Rainbow trout were anaesthetised in a solution of benzocaine (ethyl-*p*-aminobenzoate; 0.1 g l^{-1}) cooled to 10°C. After cessation of breathing movements, the fish were transferred to an operating table and the gills were irrigated with the same anaesthetic solution throughout the surgery. For experiments assessing the effects of ET-1 on plasma catecholamine levels, a single indwelling polyethylene cannula (Clay-Adams PE 50) was inserted into the dorsal aorta according to the basic method of Soivio et al. (1975). For continuous measurements of blood respiratory variables using an extracorporeal arterial blood loop, a lateral incision (~2 cm in length) was made at the level of the caudal peduncle approximately 4 mm below the lateral line. The caudal vein and artery were cannulated in the anterograde and retrograde directions, (Clay-Adams PE 50 polyethylene tubing) using standard surgical procedures (Axelsson and Fritsche 1994). The incision was sutured using a running stitch and both cannulae were then secured to the body wall with silk ligatures. A small (~1 cm) ventral incision was made to expose the pericardial cavity and the pericardium was dissected to expose the bulbus arteriosus. To enable measurement of $\dot{Q}$, a 3S or 4S ultrasonic flow probe was placed non-occlusively around the bulbus (see above). Small (1 cm^2) brass plates were stitched to the external surface of each operculum to allow the measurement of ventilation amplitude using an impedance converter (Peyraud and Ferret-Bouin 1960). After surgery, fish were placed into individual opaque acrylic boxes supplied with flowing, aerated water where they were allowed to recover for approximately 24 h before experimentation.

In situ perfusion experiments

A pithed perfused head preparation of dogfish, externally irrigated with seawater and internally perfused with saline at 12–14°C was employed, following the methods of Part et al. (1998). Irrigation rate (via external recirculation of aerated seawater entering the spiracles) was set to 2.0 l kg^{-1} min^{-1} and perfusion rate via the cardiac pump was set to 20 ml kg^{-1} min^{-1} in accord with values measured in vivo (Table 1). Perfusate PCO_2 was set to 2.3 mmHg, and perfusate trimethylamine oxide concentration was set to 85 mmol l^{-1}. All other experimental details, including composition of the perfusion saline, were identical to those described by Part et al. (1998). A three-way valve on the inflow cannula, after the Windkessel and close to the point of ventral aortic cannulation, served for injections of agonists and measurement of input pressure. The latter was corrected for pressure loss in the cannula by measurement of cannula resistance at the end of the experiment.

Table 1 Selected respiratory variables and circulating catecholamine concentrations in untreated (pre-injected) rainbow trout (*Oncorhynchus mykiss*) or dogfish (*Squalus acanthias*). Values shown are means ±1 SEM; *n* in parentheses (*pHa* arterial pH, f_R respiration frequency, PaO_2 arterial partial pressure of O_2, $PaCO_2$ arterial partial pressure of CO_2)

	Rainbow trout	Dogfish
PaO_2 (mmHg)	106.0 ± 3.4 (28)	126.4 ± 7.7* (11)
$PaCO_2$ (mmHg)	2.69 ± 0.16 (28)	1.05 ± 0.07* (11)
pHa	7.84 ± 0.02 (28)	7.89 ± 0.04 (11)
[a]Vent. Amp. (cm or mmHg)	0.35 ± 0.03 (14)	1.5 ± 0.2 (11)
f_R (min^{-1})	73.4 ± 2.4 (14)	33.1 ± 0.9* (11)
[Noradrenaline] (nmol l^{-1})	2.85 ± 1.38 (8)	1.18 ± 0.28 (9)
[Adrenaline] (nmol l^{-1})	0.95 ± 0.24 (8)	1.87 ± 0.21 (9)

[a]In trout, ventilation amplitude (Vent. Amp.) was assessed by monitoring opercular impedance changes; in dogfish, Vent. Amp. was assessed by monitoring pressure changes in the spiracle. Owing to the different methods of measurement, Vent. Amp. values for dogfish and trout were not compared statistically

*Indicates a statistically significant difference ($P < 0.05$) between the two species

Experimental protocol

Rainbow trout

Series I – effects of ET-1 on cardio-respiratory variables

Experiments were performed using extracorporeal arterial blood shunts (Thomas 1994; Perry and Gilmour 1996) that permitted continuous and simultaneous measurements of pHa, PaO_2 and $PaCO_2$. In the trout, this was achieved by pumping (peristaltic pump; 0.40 ml min^{-1}) blood from the caudal artery through pH, PCO_2 and PO_2 electrodes connected in series and returning it to the fish via the caudal vein cannula. The extracorporeal shunt contained approximately 1.0 ml of blood representing less than 3% of the total blood volume. Ventilatory and cardiovascular variables were recorded concurrently (see below).

Prior to experimentation, the extracorporeal shunt was rinsed for 20–30 min with a solution of ammonium heparin (540 IU ml^{-1}) to prevent blood from clotting in the tubing and electrode chambers. After starting the extracorporeal circulation, a period of approximately 10–15 min was required to achieve stable baseline recordings of pHa, $PaCO_2$ and PaO_2. Upon stabilisation, experiments commenced with a 10-min period of baseline recording followed by an injection (666 pmol kg^{-1} using a volume of 0.1 ml kg^{-1}) of trout ET-1 (Wang et al. 1999) or Cortland saline (Wolf 1963) into the dorsal aorta cannula; cardio-respiratory variables were monitored for a further 60 min. The dose and route of administration of ET-1 used in this study was chosen on the basis of previous experiments that showed such a protocol could elicit pronounced systemic or branchial vasoconstriction (Olson et al. 1991; Hoagland et al. 2000).

In separate groups of fish, bovine carbonic anhydrase (5 mg ml^{-1} dissolved in saline; $n = 7$) or Cortland saline (control group; $n = 7$) was injected into the caudal vein cannula 30 min prior to injection of ET-1.

Series II – effects of ET-1 on plasma catecholamine levels

These experiments were performed on fish surgically fitted only with dorsal aortic cannulae. After withdrawing a pre-injection

blood sample (0.4 ml), fish were injected via the arterial cannula with 666 pmol kg^{-1} of trout ET-1 (injected volume = 0.1 ml kg^{-1}) or 0.1 ml kg^{-1} of Cortland saline. Additional blood samples were taken 5 min, 10 min, 15 min, 30 min and 60 min after injection. All samples were centrifuged immediately (12,000 rpm for 1 min) and the plasma was placed in liquid N_2 and then stored at –80°C until subsequent analysis of catecholamine levels.

Dogfish

Series I – effects of ET-1 on cardio-respiratory variables and plasma catecholamine levels

To measure arterial blood gases (see above), an extracorporeal shunt was established by pumping (0.55 ml min^{-1}) blood from the coeliac artery (via the upstream cannula) through pH, PCO_2 and PO_2 electrodes connected in series and returning it to the fish via the downstream cannula in the coeliac artery. The extracorporeal shunt contained approximately 1.0 ml of blood representing less than 1% of the total blood volume. All other details of the shunt were as described for rainbow trout (see above) except that ET-1 was injected into the caudal vein. Cardiovascular and ventilatory variables were assessed simultaneously (see below). Blood samples (0.4 ml) were withdrawn from the caudal vein cannula before and after (5 min, 10 min, 15 min, 30 min, 60 min) ET-1 injection to measure plasma catecholamine levels.

Series II – effects of ET-1 on branchial vascular resistance in situ

After establishing stable input/perfusion pressure under conditions of constant flow (20 ml min^{-1} kg^{-1} body weight), a bolus of trout ET-1 (666 pmol kg^{-1}) was delivered to the branchial circulation via a three-way valve in the perfusate delivery line. Input pressure was monitored for a further 10-min period.

Analytical procedures

Arterial blood pH, PCO_2 and PO_2 were monitored using Radiometer or Cameron Instruments (CO_2, O_2) and Metrohm (pH) electrodes housed in thermostatted cuvettes and connected to a blood gas analyser (Cameron Instruments). The O_2 electrode was calibrated by pumping (using the peristaltic pump of the extracorporeal shunt) a zero solution (2 g l^{-1} sodium sulphite) or air-saturated water continuously through the electrode sample compartments until stable readings were recorded. The CO_2 electrode was calibrated in a similar manner using mixtures of 0.5% and 1.0% CO_2 in air that were provided by a gas mixing flowmeter (Cameron Instruments). The pH electrode (Metrohm Model 6.0204.100) was calibrated using precision buffers. The CO_2, O_2 and pH electrodes were calibrated prior to each experiment.

In trout, ventilation was assessed by monitoring opercular impedance changes using a custom-built impedance converter and amplifier. Ventilation amplitude (Vent. Amp.) was determined after conversion of the impedance data to linear opercular deflections (in cm) through appropriate calibration (performed by manually displacing the opercular covers known distances on euthanised animals). In dogfish, Vent. Amp. was assessed by monitoring pressure changes in the spiracle associated with each breathing cycle. The spiracle catheter was filled with seawater, connected to a pressure transducer (Bell and Howell) and linked to an amplifier (Harvard Biopac DA 100). The pressure transducer was calibrated daily against a static column of water.

The dorsal aortic (trout) or caudal artery (dogfish) cannula was flushed with heparinised saline (100 IU ml^{-1}) to prevent clotting and then connected to a pressure transducer (Bell and Howell) that was pre-calibrated against a static column of water. Analog blood pressure signals were measured using Harvard Biopac amplifiers (DA 100). Cardiac output was determined by attaching the ultrasonic flow probe to a Transonic T106 single channel blood flow meter.

All analog signals (blood gases, blood and ventilation pressures, impedance values and $\dot{Q}$) were converted to digital data by interfacing with a data acquisition system (Biopac Systems) using Acknowledge data acquisition software (sampling rate set at 10 Hz) and a Pentium PC. Thus, continuous data recordings were obtained for mass-specific $\dot{Q}$, cardiac frequency (f_H; automatic rate calculation from the pulsatile $\dot{Q}$ trace), cardiac stroke volume (H_{SV}; $\dot{Q}/f_H$), ventilation frequency (f_R; automatic rate calculation from the raw impedance/ventilation pressure traces), Vent. Amp. (trout; the difference between maximum and minimum impedance values, dogfish; the difference between maximum and minimum ventilation pressures), mean blood pressures and systemic vascular resistance (R_S; mean $P_a/\dot{Q}$).

Plasma adrenaline and noradrenaline concentrations were determined on alumina-extracted samples (200 µl) using high-pressure liquid chromatography (HPLC) with electrochemical detection. The HPLC consisted of a Varian Star 9012 solvent delivery system (Varian Chromatography Systems, Walnut Creek, Calif.) coupled to a Princeton Applied Research 400 electrochemical detector (EG and G Instruments, Princeton, N.J.). The extracted samples were passed through an Ultratechsphere ODS-C_{18} 5 µm column (HPLC Technology Ltd., Macclesfield, UK) and the separated amines were integrated with the Star Chromatography software program (version 4.0, Varian). Concentrations were calculated relative to appropriate standards and with 3,4-dihydroxybenzylamine hydrobromide (DHBA) as an internal standard in all determinations.

Statistical analyses

All data are represented as means ± 1 SEM unless otherwise stated. Absolute or percentage changes in measured and calculated variables were determined by subtracting the value at the injection point (10 min) from all data points. Data (absolute or absolute changes only) were analysed using a repeated measures two-way ANOVA followed by Bonferroni's multiple comparison. Statistical comparisons between trout and dogfish were carried out, where appropriate, by means of an unpaired Student's *t*-test. P values < 0.05 were considered to be statistically significant. Calculations were performed using the SigmaStat (SPSS; version 2.03) software package.

Results

Cardio-respiratory variables and plasma catecholamine levels in resting (pre-treated) trout and dogfish are depicted in Tables 1 and 2. With respect to respiratory parameters (Table 1), dogfish displayed several notable statistically significant differences from trout including greater PaO_2 (+20.4 mmHg), lower $PaCO_2$ (–1.64 mmHg) and lower f_R (–40.3 min^{-1}). For the cardiovascular data (Table 2), dogfish exhibited lower f_H (–53.8 min^{-1}) and arterial blood pressure (P_a) (–12.1 mmHg), but greater H_{SV} (+0.7 ml). Owing to these large difference in resting values and their relatively high variance in comparison to the small magnitude of several of the changes induced by ET-injection, all subsequent data are presented either as percentage (cardiovascular and ventilation data) or absolute (blood gases) changes from the final pre-injection values.

Table 2 Cardiovascular variables in untreated (pre-injected) rainbow trout (*Oncorhynchus mykiss*) or dogfish (*Squalus acanthias*). Values shown are means ±1 SEM; *n* in parentheses. ($\dot{Q}$ cardiac output, f_H cardiac frequency, H_{SV} cardiac stroke volume, P_a arterial blood pressure, R_S systemic vascular resistance)

	Rainbow trout	Dogfish
$\dot{Q}$ (ml min^{-1} kg^{-1})	22.7 ± 2.2 (16)	18.5 ± 1.6 (12)
f_H (min^{-1})	72.1 ± 1.5 (15)	18.3 ± 1.2* (12)
H_{SV} (ml kg^{-1})	0.30 ± 0.03 (15)	1.0 ± 3.8* (12)
P_a (mmHg)	28.5 ± 1.3 (25)	16.4 ± 1.7* (11)
R_S (mmHg ml^{-1} min^{-1} kg^{-1})	1.6 ± 0.2 (16)	1.3 ± 0.2 (11)

*Indicates a statistically significant difference ($P < 0.05$) between the two species

Effects of ET-1 on cardiovascular and ventilatory variables

To ensure that ET-1 was eliciting branchial vasoconstriction in dogfish, input pressure was monitored in saline-perfused head preparations before and after bolus injections. Input pressure increased from 33.4 ± 1.6 mmHg to a maximum value of 39.0 ± 1.9 mmHg after ET-1 injection, a significant 17% increase in the input pressure. Perfusate outflow from the dorsal aorta was abolished (owing to the vasoconstriction) after ET-1 injection and thus it was not possible to calculate changes in branchial vascular resistance.

In both species, injection of ET-1 caused a marked reduction in $\dot{Q}$ (Fig. 1A, B) altough the temporal responses were widely different. In trout (Fig. 1A), $\dot{Q}$ fell rapidly within 10 min of injection (maximal change = 44% or –10.1 ml min^{-1} kg^{-1} after 8 min) and then gradually returned to resting levels over the ensuing 20 min. In dogfish (Fig. 1B), the response was tri-phasic; $\dot{Q}$ initially decreased (39% or –7.7 ml min^{-1} kg^{-1} after 6 min), recovered after 28 min, and then declined again for the duration of the experiment. In trout, the effects of ET-1 on $\dot{Q}$ were mediated exclusively by transient reductions in f_H by as much as 25% (20 min^{-1}; Fig. 1C); H_{SV} was not affected significantly (Fig. 1E). In contrast, the effects of ET-1 on $\dot{Q}$ in dogfish were mediated both by transient reductions in f_H (Fig. 1D) and persistent decreases in H_{SV} (Fig. 1F).

The effects of ET-1 on R_S varied markedly between the species (Fig. 1G, H). In rainbow trout (Fig. 1G), R_S was increased rapidly (within 2 min) and at the height of the response (10 min post-injection) was increased by 95% (+1.4 mmHg ml^{-1} min^{-1} kg^{-1}. R_S then declined for the duration of the experiment and was restored to pre-injection levels after 34 min. Dogfish, on the other hand, displayed a bi-phasic response in which R_S was decreased (~32%) within the initial 10 min after ET-1 injection and then was significantly increased (peak response = 69% increase) during the final 30 min of monitoring (Fig. 1H). The apparent transient increase in R_S in saline-injected dogfish was not statistically significant (Fig. 1H).

Owing to the different patterns of changes in $\dot{Q}$ and R_S in dogfish and trout, the effects of ET-1 on P_a also were markedly different (Fig. 1I, J). In trout, P_a was unaffected by ET-1 for ~40 min but then displayed a gradual decline for the last 20 min of the measurement period. In dogfish, ET-1 injection caused a pronounced transitory reduction in P_a (58% or –10.1 mmHg after 6 min) that was rapidly restored and thereafter remained constant (Fig. 1J).

The effects of ET-1 on Vent. Amp. and f_R are depicted in Fig. 2. In both species, ET-1 injection caused marked hyperventilatory responses although of varied duration. In trout, the increase in Vent. Amp. (as measured by changes in impedance) was temporary whereas in dogfish, the increase in Vent. Amp. (as measured by pressure changes) was persistent. Similarly, f_R was increased only initially in trout (maximal increase = 24% or 16 min^{-1}) whereas in dogfish, the increase in f_R (maximal increase = 22% or 7 min^{-1}) was maintained through the period of experimentation.

In both species, ET-1 caused a rapid (trout – 4 min; dogfish – 10 min) reduction in $P\mathrm{aO_2}$ (Fig. 3). In trout, the greatest effect (–57.3 mmHg) was observed 24 min after injection. Although there was a trend for recovery in trout, (Fig. 3A), $P\mathrm{aO_2}$ was still significantly reduced (–26.6 mmHg) at the conclusion of the experiment (60 min post-injection). In dogfish, the response was triphasic consisting of an initial decline (maximum effect = –28.0 mmHg) followed by rapid restoration and then a persistent reduction (Fig. 3B). The effects of ET-1 on $P\mathrm{aCO_2}$ were not synchronised with the changes in $P\mathrm{aO_2}$ and the responses were markedly different in trout and dogfish (Fig. 3C, D). In trout, $P\mathrm{aCO_2}$ was increased transiently by ~1.0 mmHg but the onset of the response was delayed and occurred 12 min after ET-1 injection (Fig. 3C). In contrast, $P\mathrm{aCO_2}$ remained more or less constant in dogfish after injection of ET-1 and was increased only slightly (~0.1 mmHg) after 60 min (Fig. 3D). The relationships between changes in $P\mathrm{aO_2}$ and $P\mathrm{aCO_2}$ after ET-1 injection are illustrated in Fig. 4. The absence of any relationship between $\Delta P\mathrm{aO_2}$ and $\Delta P\mathrm{aCO_2}$ in trout (Fig. 4A) during the initial 12 min after injection is clearly evident while in dogfish (Fig. 4B), these two variables were not correlated at any point after ET-1 treatment. In both species, ET-1 injection caused a decrease in pHa (ΔpHa in trout = –0.20 after 34 min; ΔpHa in dogfish = –0.08 after 58 min) that mirrored the changes in $P\mathrm{aCO_2}$ (Fig. 3). The injection of exogenous bovine CA to the circulation of trout eliminated the rise in $P\mathrm{aCO_2}$ that normally followed ET-1 injection (Fig. 5A); $P\mathrm{aO_2}$ still declined in CA-treated trout although the response was somewhat attenuated (Fig. 5B).

In both species, ET-1 injection was associated with increases in plasma catecholamine levels (Fig. 6). In each case, the predominant circulating catecholamine after ET-1 treatment was adrenaline (data not shown).

Discussion

Several previous studies have reported on the in vivo cardiovascular effects of ET-1 in teleosts including

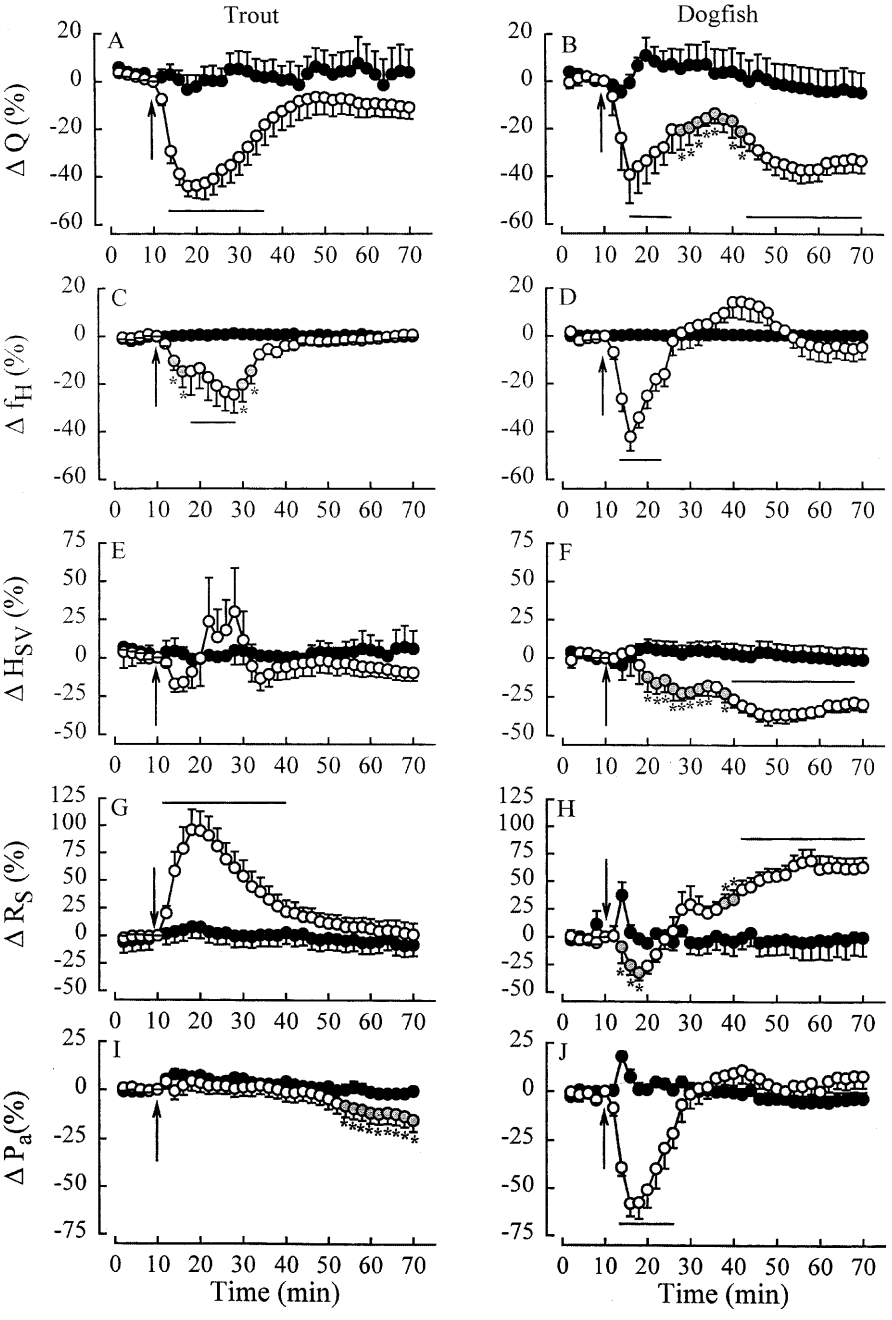

Fig. 1A–J The effects of a bolus injection of 666 pmol kg^{-1} of trout endothelin-1 (ET-1) (*unfilled circles*) or saline (*filled circles*) on changes in cardiac output ($\Delta \dot{V}b$), cardiac frequency (Δf_H), cardiac stroke volume (ΔH_{SV}), systemic vascular resistance (ΔR_S) and arterial blood pressure (ΔP_a) in freshwater rainbow trout [$n = 6$ (controls) and $n = 10$ (ET-1-injected)] or marine dogfish [$n = 6$ (controls) and $n = 6$ (ET-1-injected)]. The *left panels* (**A**, **C**, **E**, **G**, **I**) correspond to rainbow trout and the *right panels* (**B**, **D**, **F**, **H**, **J**) correspond to dogfish. In each panel, a *vertical arrow* indicates the time of injection. Significant differences ($P < 0.05$) from both the final pre-injection values (time = 10 min) and from the saline-injected fish are indicated by *horizontal lines*; significant differences ($P < 0.05$) from only the final pre-injection values (time = 10 min) are represented by *asterisks* and *shaded symbols*

rainbow trout (Olson et al. 1991; Sundin and Nilsson 1998; le Mevel et al. 1999; Hoagland et al. 2000) and Atlantic cod, *Gadus morhua* (Stenslokken et al. 1999). This is the first study, however, to address the in vivo effects of ET-1 in an elasmobranch species. Furthermore, despite the presence of ET-1 receptors in the gill (Lodhi et al. 1995; Evans and Gunderson 1999) and the known effects of ET-1 on reducing gill functional surface area (Sundin and Nilsson 1998), only a single study (Stenslokken et al. 1999) has addressed the potential impact on respiratory gas transfer. In that study, blood PO_2 was the only respiratory variable that was monitored. Thus, this paper presents the first detailed description of the effects of ET-1 induced branchial vasoconstriction on gas transfer across the fish gill.

In previous studies, a range of doses (6.3–2500 pmol kg^{-1}) and differing methods of intravascular administration (bolus injection versus continuous infusion) were used to assess the cardiovascular effects of ET-1 in fish (Olson et al. 1991; Sundin and Nilsson 1998; Stenslokken et al. 1999; le Mevel et al. 1999; Hoagland et al. 2000). In the present investigation, we opted to use a bolus injection into the dorsal aorta (trout) or caudal vein (dogfish) and a high dose of trout ET-1 (666 pmol kg^{-1}); an identical protocol was shown previously to markedly increase gill resistance in vivo in rainbow trout (Hoagland et al. 2000). Despite the ability

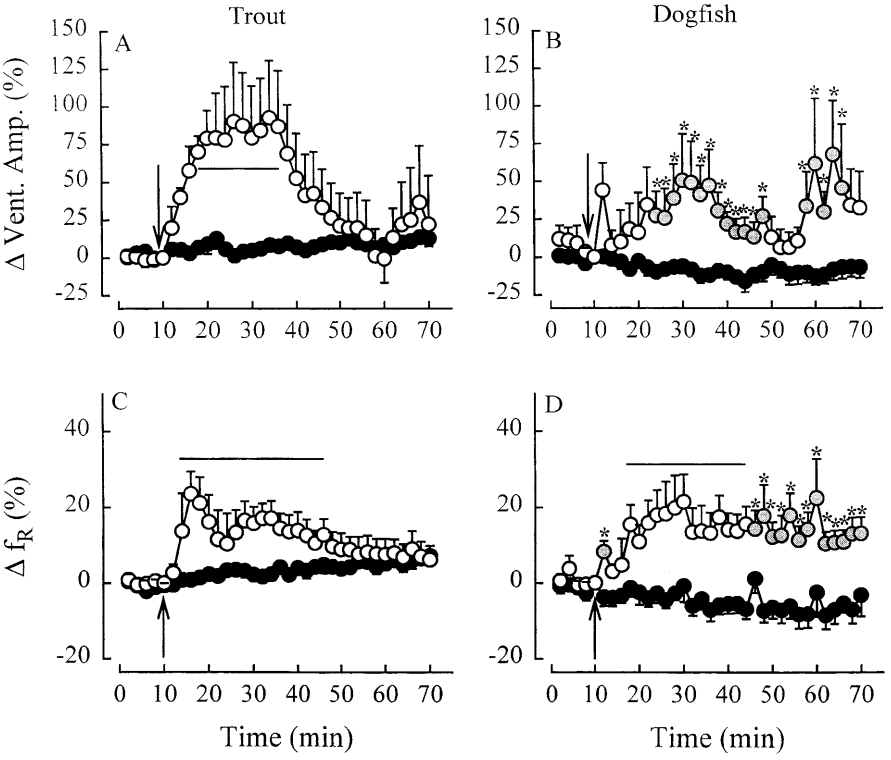

Fig. 2 The effects of a bolus injection of 666 pmol kg^{-1} of trout ET-1 (*unfilled circles*) or saline (*filled circles*) on changes in ventilation amplitude (ΔVent. Amp.) or ventilation frequency (f_R) in **A**, **C** freshwater rainbow trout [$n = 8$ (controls) and $n = 6$ (ET-1-injected)] or **B**, **D** marine dogfish [$n = 5$ (controls) and $n = 5$ (ET-1-injected)]. In each panel, a *vertical arrow* indicates the time of injection. Significant differences ($P < 0.05$) from both the final pre-injection values (time = 10 - min) and from the saline-injected fish are indicated by *horizontal lines*; significant differences ($P < 0.05$) from only the final pre-injection values (time = 10 min) are represented by *asterisks* and *shaded symbols*

of the gill to clear 50% of circulating ET-1 in a single pass (Olson 1998) and an estimated half-time for circulating ET-1 of ~3 min in trout (Olson et al. 1997), the initial circulating levels in the present study probably were 100–1000× greater than ET-1 concentrations reported for fish plasma (Uemura et al. 1991). However, as in mammals, the level of ET-1 naturally circulating in the plasma of fishes (< 4 pmol l^{-1}; Uemura et al. 1991) is likely well below the concentration required to elicit vascular constriction (> 1 nmol l^{-1}; Poder et al. 1991). Because ET-1 is found concentrated within fish gill neuroendocrine cells (Goniakowska-Witalinska et al. 1995; Zaccone et al. 1996; Mauceri et al. 1999), there is the likelihood of its localised release into the microcirculation whereby much higher (physiologically active) levels could be achieved. Thus, although the doses of ET-1 used in the present study likely produced abnormally high levels (at least initially) in the general circulation, such concentrations could be achieved in localised areas of the gill or systemic circulation. Regardless, a key aspect of this study was to assess the effects of vasoconstriction-mediated reductions in functional gill surface area on gas transfer. Thus, ET-1 was used as a tool to evoke appropriate adjustments in gill perfusion. As such, achieving physiologically relevant levels of ET-1 in the circulation was less important than ensuring that in both species, the injections evoked sufficient changes in branchial flow patterns to significantly reduce diffusion conductance. Ideally, a range of doses of ET-1 would have been used to establish the levels required to evoke maximal or near maximal responses in both species. For practical reasons (insufficient quantities of ET-1 and time/animal constraints at Bamfield Marine Station), however, this was not feasible. Thus, we opted to use a single dose (666 pmol kg^{-1}) for both species. In trout, higher doses of ET-1 (e.g. 2500 pmol kg^{-1}; Olson et al. 1991) may induce mortality.

Two other methodological issues are: (1) the differing sites of ET-1 injection in the two species, and (2) the use of homologous versus heterologous peptide. In trout, ET-1 was injected into the dorsal aorta whereas in the dogfish, the caudal vein was the route of administration. As discussed by Hoagland et al. (2000), owing to dilution effects, injection into the arterial (post-gill) circulation is likely to produce a smaller effect on the branchial vasculature than its injection into the venous (pre-gill) circulation. Thus, despite similar injected doses, the branchial receptors in dogfish were likely exposed to higher levels of ET-1. Finally, because ET-1 has not yet been sequenced in any elasmobranch, trout ET-1 was used in both species. Thus, there is a possibility that the potency of the peptide varied between the trout and the dogfish. Although the structure of ET has been remarkably conserved through evolution (Wang et al. 1999), a substitution of a mere three conservative amino acids results in the human peptide being at least an order of magnitude more potent than the trout peptide in constricting trout blood vessels (Wang et al. 1999). For these reasons, quantitative comparisons of the effects of ET-1 in trout and dogfish are not valid and indeed are not relevant to the overall conclusions of this study. Rather, the more critical issue was whether in both species, ET-1 was decreasing gill diffusion conductance; clearly this was the case (see below).

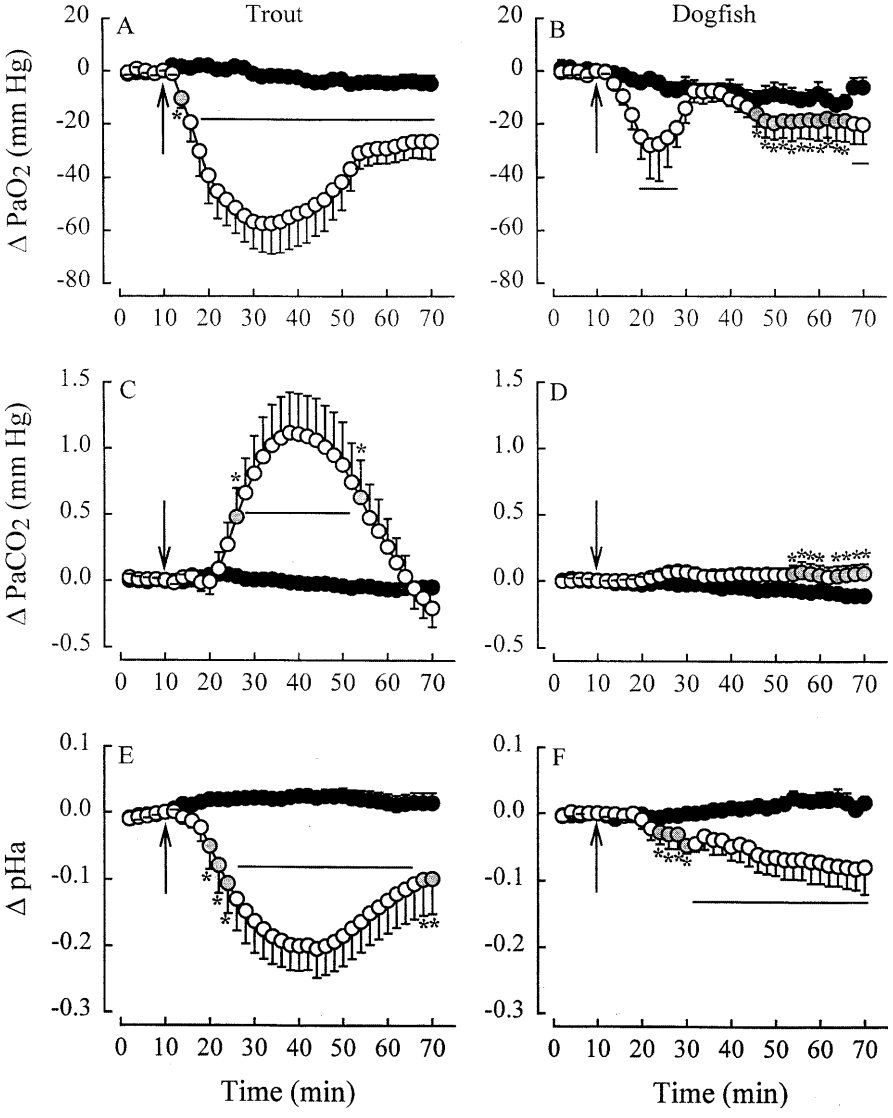

Fig. 3 The effects of a bolus injection of 666 pmol kg^{-1} of trout ET-1 (unfilled circles) or saline (filled circles) on changes in arterial blood oxygen partial pressure (ΔP_aO_2), arterial blood carbon dioxide partial pressure (ΔP_aCO_2) and arterial blood pH (pHa) in freshwater rainbow trout (**A**, **C**, **E**) [$n = 14$ (controls) and $n = 8$ (ET-1-injected)] or marine dogfish (**B**, **D**, **F**) [$n = 6$ (controls) and $n = 5$ (ET-1-injected)]. In each panel, a *vertical arrow* indicates the time of injection. Significant differences ($P < 0.05$) from both the final pre-injection values (time = 10 min) and from the saline-injected fish are indicated by *horizontal lines*; significant differences ($P < 0.05$) from only the final pre-injection values (time = 10 min) are represented by *asterisks* and *shaded symbols*

Cardiovascular effects of ET-1 in trout and dogfish

Trout

The cardiovascular effects of ET-1 injection in trout were similar to those reported previously for teleosts (Stenslokken et al. 1999; le Mevel et al. 1999; Hoagland et al. 2000). However, unlike prior studies that demonstrated pressor (le Mevel et al. 1999), depressor (Olson et al. 1991) or mixed pressor/depressor (Hoagland et al. 2000) responses to similar or lower doses of ET-1 in trout, P_a in the present study remained virtually constant except for a slight decrease (~4 mmHg; 14%) during the final 12 min of recording. The net effect of ET-1 on P_a predominantly reflects the simultaneous changes to R_S, R_G and $\dot{Q}$. Owing to a pronounced branchial vasoconstriction, R_G was increased (Hoagland et al. 2000) and caused a lowering of P_a while increasing ventral aortic blood pressure (P_{VA}; not measured in the present study). A concomitant increase in R_S promoted an elevation of P_a that was opposed by a simultaneous decrease in $\dot{Q}$. Consequently, the net effect of these changes on P_a reflected the magnitude and the time course of the different responses. Clearly, in the present study, the increase in R_G and decrease in $\dot{Q}$ were sufficient to counteract the increase in R_S. In contrast to a previous report (Hoagland et al. 2000), the decrease in $\dot{Q}$ was mediated exclusively by adjustments to f_H; H_{SV} was not affected. The effect of ET-1 on $\dot{Q}$ presumably was a barostatic response and was unlikely to have been mediated by any direct effect of ET-1 on the heart itself. Although ET-1 was shown to have positive inotropic effects in the mammalian heart (Ishikawa et al. 1988), it has no known direct effects on cardiac function in the isolated perfused trout heart (Olson et al. 1991).

In mammals, ET-1 is a potent activator of catecholamine (Hinojosa-Laborde and Lange 1999) and corticosteroid (Nussdorfer et al. 1997) release from the adrenal medulla and cortex, respectively. Although a previous study (le Mevel et al. 1999) demonstrated that

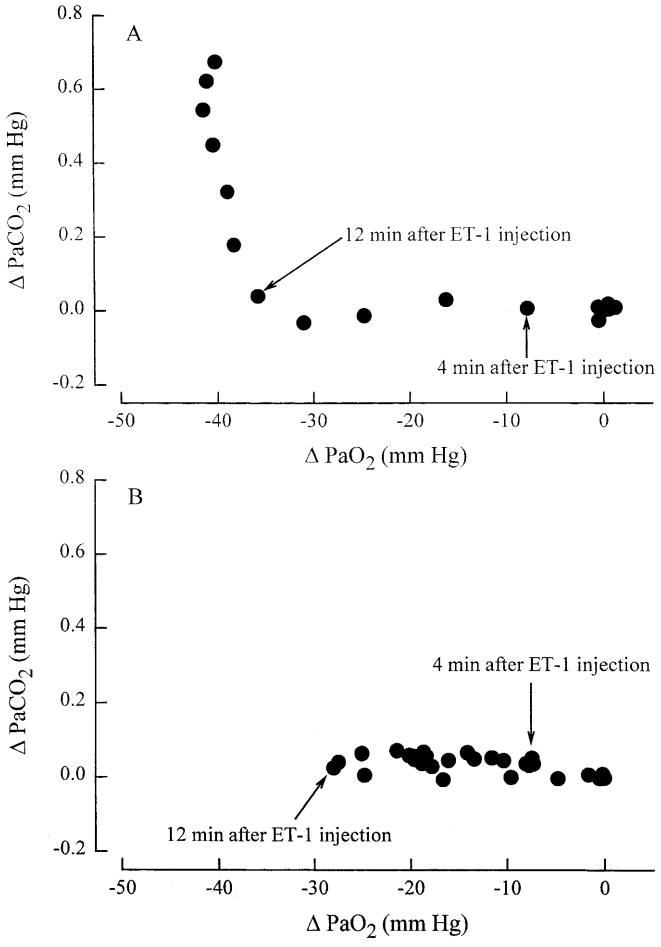

Fig. 4 The relationships between the changes in arterial blood PO_2 (ΔPaO_2) and PCO_2 ($\Delta PaCO_2$) after a bolus injection of 666 pmol kg^{-1} of trout ET-1 in **A** rainbow trout (*Oncorhynchus mykiss*) and **B** dogfish (*Squalus acanthias*). See text for further details. Mean values from Fig. 3 are re-plotted without their error bars, for clarity

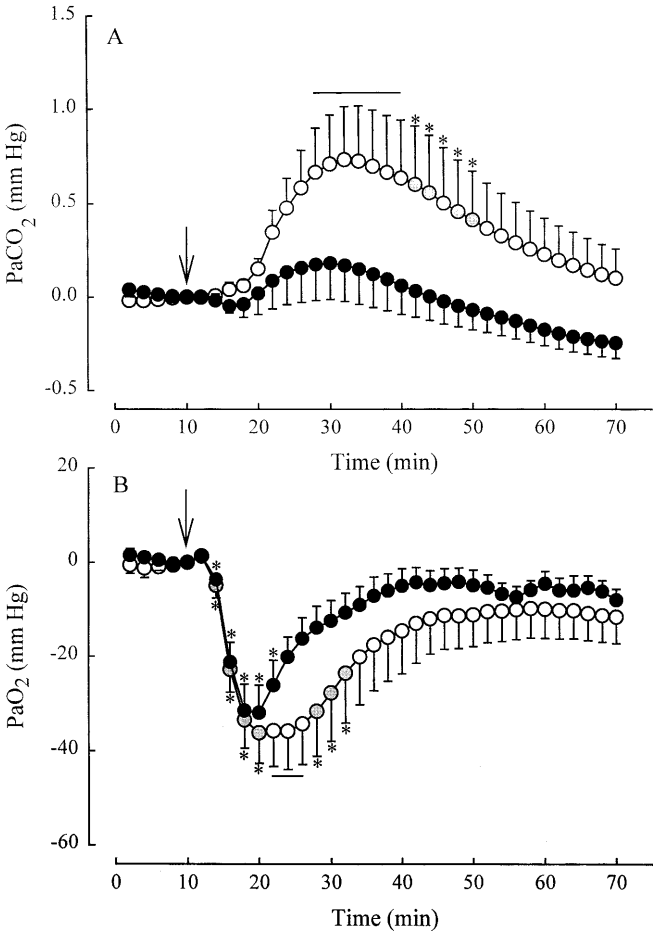

Fig. 5 The effects of a bolus injection of 666 pmol kg^{-1} of trout ET-1 on changes in **A** arterial blood carbon dioxide partial pressure ($\Delta PaCO_2$) or **B** arterial blood oxygen partial pressure (ΔPaO_2) in freshwater rainbow trout pre-treated with bovine carbonic anhydrase (CA) (*filled circles*; $n=8$) or saline [controls (*unfilled circles*; $n=8$)]. In each panel, a *vertical arrow* indicates the time of injection. Significant differences ($P<0.05$) from both the final pre-ET-1 injection values (time = 10 min) and from the corresponding values in the control fish are indicated by *horizontal lines*; significant differences ($P<0.05$) from only the pre-ET-1 injection values are represented by *asterisks* and/or *shaded symbols*

low doses of ET-1 (22–86 pmol kg^{-1}) did not promote an elevation of circulating cortisol levels in trout, the present results demonstrated a significant increase in circulating catecholamine concentrations. Further studies are required to determine the source of the circulating catecholamines (release from chromaffin cells versus neuronal spillover) and whether the effects are direct or indirectly caused by the cardiovascular/respiratory adjustments accompanying ET-1 injection.

Dogfish

Recently, ET_B receptors were identified in the gills of dogfish on the basis of specific ligand binding (Evans and Gunderson 1999). The present study clearly shows that, as in teleost fish, activation of these receptors can evoke pronounced branchial vasoconstriction. However, in marked contrast to the branchial vasculature, and in contrast to those teleosts that have been examined (trout, cod), the systemic vasculature of dogfish displayed an initial vasodilatory response to ET-1. This response resembled the transient systemic vasodilatation that accompanies ET-1 infusion in mammals (Schiffrin and Touyz 1998) and which is thought to be linked to nitric oxide release from vascular endothelial cells after activation of ET_B receptors. Owing to the combined effects of systemic vasodilatation, branchial constriction and decreased $\dot{Q}$, P_a initially was markedly lowered after injection of ET-1. The delayed rise in R_S presumably was caused by activation of vascular smooth muscle $ET_{A/B}$ receptors. In mammals, activation of the ET_A receptors of vascular smooth muscle is thought to be the predominant cause of the systemic vasoconstriction. In dogfish, constriction of the ventral aorta in vitro appears to be mediated exclusively by ET_B receptors (Evans et al. 1996). Thus, ET_B receptors (if also present in the systemic

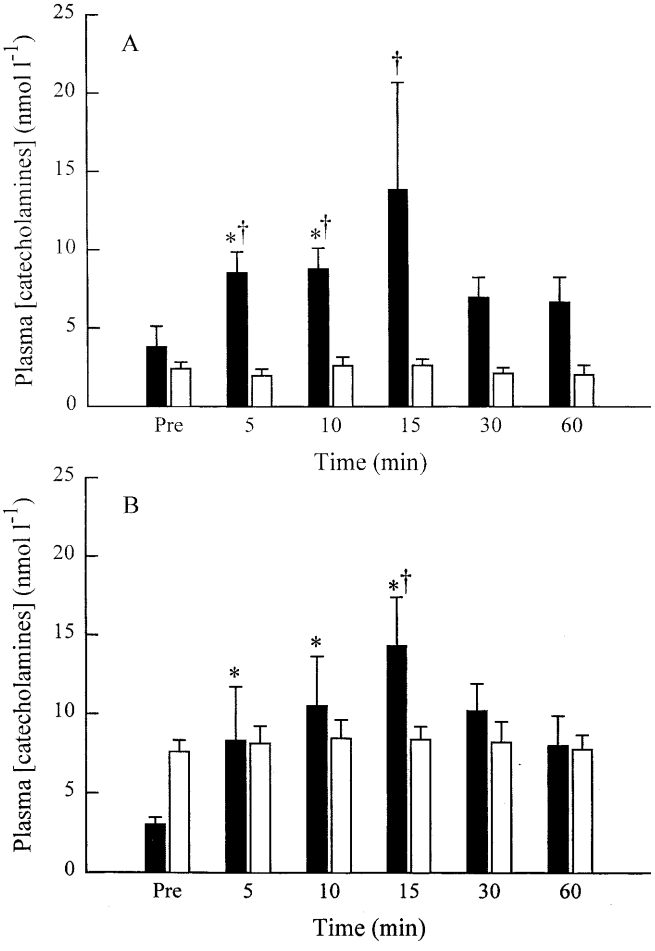

Fig. 6 The effects of a bolus intra-arterial injection of 666 pmol kg^{-1} of trout ET-1 (*filled bars*) or saline (*unfilled bars*) on total plasma catecholamine (adrenaline plus noradrenaline) levels in **A**) rainbow trout (*Oncorhynchus mykiss*; $n=8$) or **B** dogfish (*Squalus acanthias*; $n=9$). * indicates a significant difference ($P<0.05$) from the corresponding pre-injection (*Pre*) value; † indicates a significant difference ($P<0.05$) from the corresponding control value (saline-injected fish). All data are shown as means ±1 SEM

vasculature) may also play an important role in mediating the delayed systemic vasoconstriction that was observed in dogfish. During the period of systemic vasoconstriction, arterial blood pressure remained constant and this presumably reflected the counteracting influences of decreased $\dot{Q}$ and elevated R_G (not measured in vivo in this study). As in trout, plasma catecholamines were elevated after ET-1 injection. Owing to the absence of sympathetic innervation to the dogfish heart and the presumed importance of circulating catecholamines in modulating cardiovascular function in elasmobranchs (Butler and Metcalfe 1988), the increases in plasma levels may have had more impact than in trout.

Respiratory effects of ET-1 in trout and dogfish

Gas transfer across the fish gill is influenced by a host of factors (reviewed by Gilmour 1997) but for the purposes of this discussion, the two critical variables are diffusion conductance (G_{DIFF}) and blood transit time. Diffusion conductance is determined by the functional surface area that is available for gas transfer, the blood-to-water diffusion distance and the permeability of the multi-layered lamellar epithelium to O_2 and CO_2.

Trout

Current models for gas transfer across the teleost gill contend that there are greater diffusion limitations imposed upon CO_2 excretion than O_2 uptake (see reviews by Perry 1986; Piiper 1989; Swenson 1990; Gilmour 1997; Tufts and Perry 1998). However, the limitations on CO_2 transfer do not reflect problems of diffusion per se, but rather the requirement to first convert plasma HCO_3^- to CO_2 during blood transit time (~1–3 s). This is because the accessibility of plasma HCO_3^- to RBC CA is constrained by the relatively slow entry of HCO_3^- into the RBC via electroneutral Cl^-/HCO_3^- exchange. This so-called chemical equilibrium limitation lowers the effective time period available for CO_2 diffusion below gill transit time and is believed to be a major cause of apparent diffusion limitations for CO_2 transfer across the gill (Swenson 1990; Tufts and Perry 1998; Julio et al. 2000). Because of such chemical equilibrium limitations, branchial CO_2 transfer theoretically is expected to be highly sensitive to changes in the transit time of blood through the teleost gill. Indeed, recent data (P. Desforges, S. Harman, K.M. Gilmour and S.F. Perry, unpublished observations) have revealed a significant positive correlation ($r^2=0.77$; $n=18$) between $\dot{Q}$ and $PaCO_2$ in the absence of any effect on PaO_2 in trout subjected to acute vascular volume loading.

With this background, an explanation for the complex effects of ET-1 injection on arterial blood gases in trout can be suggested. Owing to constriction of lamellar blood channels (likely caused by pillar cell contraction), a significant proportion of the cardiac output is re-routed around the perimeter of the lamella through marginal channels (Sundin and Nilsson 1998; Stenslokken et al. 1999). The inner marginal channel is ill suited for gas transfer because it is buried within the filament. Given equivalent transit times, gas transfer across the outer marginal channel (OMC) should be no less efficient than is gas transfer across the central lamellar sheet. However, the marked increase in blood flow velocity through the OMC (Sundin and Nilsson 1998; Stenslokken et al. 1999) must drastically reduce the time available for gas equilibration thereby reducing efficiency. The mixing of large volumes of blood from the marginal channels (low PO_2, high PCO_2) with much smaller volumes of blood arising from the central vascular sheet would be expected to lower PaO_2 and raise $PaCO_2$. In trout, however, PaO_2 decreased rapidly after ET-1 injection whereas $PaCO_2$ remained constant for approximately 12 min and only thereafter

began to increase. The maintenance of constant P_aCO_2 (despite the re-routing of blood to the marginal channels) likely was caused by an associated increase in transit time through the central vascular sheet (Sundin and Nilsson 1998; Stenslokken et al. 1999) that was caused by the decreased $\dot{Q}$ coupled with a lower proportion of $\dot{Q}$ directed to central lamellar regions. Effectively, this would relieve the limiting factor for CO_2 excretion (time required for dehydration of plasma HCO_3^-) and allow a longer period of time for CO_2 equilibration across the gill. As cardiac output began to return to control levels (~10 min after ET-1 injection), chemical equilibrium limitations were re-established and when coupled with a persistent re-routing of blood to the marginal channels (as indicated by the prolonged reduction in P_aO_2), resulted in the delayed rise in P_aCO_2.

Two additional factors that may have influenced blood gases after ET-1 injection were changes in ventilation and potential changes in venous blood gas tensions. It is not clear whether the hyperventilatory response was a direct effect of ET-1 or indirectly mediated by O_2 chemoreceptors that sensed the decline in blood O_2 status. Regardless, the hyperventilation presumably served to partially ameliorate the effects of ET-1 on branchial gas transfer. Venous blood gas tensions were not measured in the present study yet they were likely affected by the changes in cardiac output (influencing tissue transit times) coupled with modifications in systemic resistance. It is unlikely, however, that changes in venous gas tensions could have significantly contributed to the measured changes in arterial blood gas tensions. Assuming constant O_2 consumption, the decreases in cardiac output would have been associated with decreases in venous O_2 concentration (C_vO_2). However, even large changes in C_vO_2 would cause only small changes in venous P_O_2 (P_vO_2) because of the high O_2 capacitance of the blood at the low P_O_2s normally occurring in the venous blood. It is also improbable that increases in venous P_CO_2 (P_vCO_2), if occurring, would have significantly influenced P_aCO_2 after ET-1 injection. First, the changes in P_aCO_2 were not synchronised with the changes in cardiac output. Second and more importantly, the increases in P_aCO_2 in trout were prevented by pre-treatment with carbonic anhydrase yet this protocol is known to have no effect on P_vCO_2 (Desforges et al. 2001).

The results of the present study are in marked contrast to a previous experiment in which P_aO_2 was unaffected by ET-1 injection in Atlantic cod (Stenslokken et al. 1999). Several factors may explain the conflicting results including dosage differences (666 pmol kg^{-1} versus 45 pmol kg^{-1}) and timing of the blood sampling (a single sample was taken 5–8 min after ET-1 injection in cod). Although an obvious loss of gill functional surface was observed by Stenslokken et al. (1999), it was argued (as in the present study) that an associated increase in the residence time of blood within the lamella was offsetting the decrease in G_{DIFF}.

Dogfish

Dogfish possess CA within their plasma (Wood et al. 1994; Henry et al. 1997) as well as extracellular CA that is bound to gill plasma membranes and oriented toward the blood (Gilmour et al. 1997). Thus, as blood flows through the gill, plasma HCO_3^- can be dehydrated within the plasma as well as within the RBC (Gilmour 1998). Although the relative importance of extracellular versus RBC CA in CO_2 excretion has not yet been established, recent data suggest that the dehydration of HCO_3^- within the plasma may contribute significantly to overall CO_2 excretion (Gilmour et al. 2001). Therefore, in comparison to the situation in rainbow trout, CO_2 excretion in dogfish would be less constrained by chemical equilibrium limitations and thus less sensitive to transit time changes imposed by adjustments to cardiac output. Moreover, CO_2 excretion would then be less sensitive to changes in diffusion conductance than O_2 uptake owing to the greater diffusivity of CO_2 across the gill (Swenson 1990). The fact that P_aCO_2 was more-or-less unaffected by ET-1 despite an obvious decrease in G_{DIFF} (as indicated by the lowering of P_aO_2) and oscillating $\dot{Q}$, is consistent with these theoretical predictions.

If the presence of extracellular CA in dogfish were indeed responsible for the insensitivity of CO_2 excretion to changes in G_{DIFF} and transit time, one would expect that injecting CA into the plasma of trout would cause trout to respond like dogfish. Indeed, this was the case because the addition of exogenous CA to the plasma of trout abolished the increase in P_aCO_2 that was normally observed after ET-1 injection. Thus, the presence of extracellular carbonic anhydrase in dogfish may confer significant protection of blood acid-base status during periods of branchial vasoconstriction. Because trout lack extracellular CA, their blood acid-base status is much more likely to be influenced by changes in transit time that accompany branchial vasoconstriction.

Acknowledgements This research was supported by NSERC Research and Equipment grants to SFP, KMG and CMW and NSF Grant No. IBN 9723306 to KO. We would like to thank all of the staff at Bamfield Marine Station, and especially the Research Coordinator, Nathan Webb, for their hospitality and their assistance. CJM and JM were recipients of NSERC and OGSST scholarships, respectively. The animals were cared for in accordance with the principles of the Canadian Council for Animal Care 'Guide to the Care and Use of Experimental Animals'.

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