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CARDIOVASCULAR AND GILL MICROCIRCULATORY EFFECTS OF ENDOTHELIN-1 IN ATLANTIC COD: EVIDENCE FOR PILLAR CELL CONTRACTION

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Summary

Endothelin-1 (ET-1) has been shown to cause a considerable increase in the vascular resistance of fish gills. In trout, recent evidence suggest that this is the result of pillar cell contraction in the gill lamellae. Using epi-illumination microscopy to observe the gill lamellae of anaesthetised Atlantic cod (*Gadus morhua*), we show that ET-1 (100 ng kg^{-1}, injected into the ventral aorta) causes an increase in pillar cell diameter, consistent with pillar cell contraction, and a shift of intralamellar blood flow from the lamellar sheet to the outer marginal channels. Simultaneously, there was an increase in ventral aortic blood pressure, a reduction in cardiac output, an increase in gill vascular resistance and a reduction in the oxygen partial pressure of venous blood. All these effects were blocked by the ET_A/ET_B receptor antagonist bosentan (5 mg kg^{-1}). Pillar cell contraction is likely to be a mechanism for matching the functional respiratory surface area with the instantaneous respiratory needs of the fish.

Key words: bosentan, cod, *Gadus morhua*, endothelin, gill, respiration, secondary lamella, vasoconstriction.

Introduction

The primary site for oxygen uptake in fish, the gill lamella, can be described as a blood-filled pillared hall, in which epithelial cells form the roof and floor, and where the pillars are made up of pillar cells. These cells restrain the lamella under the internal force of blood pressure (Laurent, 1984; Olson, 1991), and the blood flow through the lamella can be described as sheet flow (Farrell et al., 1980). The polygonal distribution of pillar cells probably serves to enhance gas exchange by scattering, squeezing and slowing down the erythrocytes passing through the lamellar sheet (Nilsson et al., 1995). However, as we shall see, the pillar cells may also play a more dynamic role in gill microcirculation.

Around the edge of each lamella run the inner and outer marginal channels, which serve as fast bypasses for the erythrocytes. Teleost fishes are either hypo- or hyperosmotic compared with the surrounding water. As a result, there is a trade-off between oxygen uptake and energetically expensive ion and water fluxes over the lamellae. It has been suggested that fish can adjust their functional respiratory surface area to match their instantaneous oxygen requirement either by varying the number of perfused lamellae (‘lamellar recruitment’; Booth, 1978, 1979) or by changing the pattern of blood flow within the lamellae (from the lamellar sheet to the marginal channels or *vice versa*) (Soivio and Hughes, 1978; Soivio and Tuurala, 1981; Farrell et al., 1980). Such changes have been attributed to increased lamellar blood pressure causing passive distension of the lamellae (Smith and Johnson, 1977; Farrell et al., 1980; Smith and Chamley-Campbell, 1981; Sundin and Nilsson, 1997).

Endothelin-1 (ET-1) is an extremely potent endogenous vasoconstrictor in many mammalian vessels (Le Monnier de Gouville et al., 1989; Masaki, 1989). ET-immunoreactivity has subsequently been found to be present in fish (Goniakowska-Witalinska et al., 1995; Zaccone et al., 1996; Masini et al., 1996, 1997), where ET-1 is an equally potent constrictor of various blood vessels (Olson et al., 1991; Poder et al., 1991; Sverdrup et al., 1994; Brown and Amer, 1997). Olson et al. (1991) showed that ET-1 produces a dose-dependent increase in the resistance to flow through isolated perfused gill arches from rainbow trout (*Oncorhynchus mykiss*) and also decreases dorsal aortic pressure in trout when injected *in vivo*.

Using epi-illumination microscopy *in vivo*, we recently found that a prebranchial injection of ET-1 causes a strong reduction in intralamellar blood flow (Sundin and Nilsson, 1998). This occurred in spite of the fact that the ET-1 injection also caused a doubling of prebranchial (ventral branchial artery) blood pressure (which should act to distend the lamellae). Simultaneously, we observed an increased rate of flow in the outer marginal channels and obtained clear visual indications of an increased diameter of the pillar cells consistent with a contraction of the pillar cells. In contrast, there were no observable changes in the diameter of the afferent and efferent filament arteries and lamellar arterioles. These observations constituted the first direct evidence for

pillar cell contraction, a conclusion supported by the finding of a high density of ET receptors in the lamellae of rainbow trout (Lodhi et al., 1995). Moreover, pillar cells contain microfilaments characteristic of smooth muscle myosin, indicating an ability to contract (Newstead, 1967; Bettex-Galland, 1973; Smith and Chamley-Campbell, 1981).

The present study was carried out *in vivo* on anaesthetised Atlantic cod *Gadus morhua*, a stenohaline saltwater species, with the aims of investigating the effects of ET-1 on gill microcirculation (using epi-illumination microscopy), cardiac output ($\dot{Q}$), heart rate, ventral and dorsal aortic blood pressures, and ventral and dorsal aortic oxygen partial pressures (P_{O_2}). In addition, we tested the possible blocking effect of bosentan, a mammalian ET receptor antagonist. A blocker of fish ET receptors would provide a valuable tool for further investigations into the cardiovascular roles of ET-1 in fish. No ET receptor blocker has been shown to inhibit the cardiovascular effects of ET-1 in fish.

Materials and methods

The experiments were carried out in July at Klubban Marine Biological Station (Uppsala University) at the Gullmarsfjord, Fiskebäckskil, on the west coast of Sweden. The Atlantic cod *Gadus morhua* L. (range 360–650 g) were obtained through local fishermen and kept in tanks (five cod per m^3) continuously supplied with sea water (10–14 °C). They were anaesthetised by adding benzocaine (NMD, Oslo) dissolved in ethanol (50 g l^{-1}) to the water to a final concentration of 40 mg l^{-1}. During surgery and subsequent experiments, the gills were irrigated with recirculating refrigerated sea water (10 °C, 400 ml min^{-1}, containing 30 mg l^{-1} benzocaine) flowing through a tube into the mouth.

A polyethylene catheter (PE50), inserted occlusively into the afferent branchial artery of the left third gill arch, was used for measurement of ventral aortic blood pressure (P_{VA}) and injections of ET-1 and bosentan. To record dorsal aortic blood pressure (P_{DA}), a 10 mm long piece of a smaller polyethylene catheter (PE10) was attached to a PE50 catheter and then inserted occlusively into the efferent branchial artery of the right third gill arch. Both catheters were filled with heparinised (100 i.u. ml^{-1}) 0.9 % NaCl and attached to Gould Statham P23 Db pressure transducers.

Cardiac output ($\dot{Q}$) was measured by fitting a cuff-type single-crystal Doppler flow probe (University of Iowa Bioengineering) around the ventral aorta (Sundin, 1995).

Finally, the outer half of the right operculum was removed (to permit microscopic observations), and the fish was placed on its side in a rectangular acrylic box mounted on the stage of a microscope. The box was equipped with a stand-pipe adjusted so that the recirculating respiratory water rose to a level that covered the fish. The animals were left for 1 h before any experiments were conducted.

To measure the oxygen partial pressure (P_{O_2}) in the ventral and dorsal aorta, an extracorporal loop was used. The loop was driven by a peristaltic pump (set at 200 µl min^{-1}). To measure ventral aortic P_{O_2}, blood was pumped from the ventral aortic catheter through a 50 µl chamber holding a Clark-type oxygen electrode (Microelectrodes Inc., Londonderry, NH, USA) and into the dorsal aorta. The direction of flow was reversed to measure dorsal aortic P_{O_2}.

The ventral aortic cannula was also used for injections of ET-1 (500 ng ml^{-1}=201 pmol ml^{-1}; Sigma) and bosentan {Ro 47-0203; 4-tert-butyl-*N*-[6(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzenesulphonamide sodium salt; 5 mg ml^{-1}=8.7 µmol ml^{-1}; a gift from Actelion Ltd, Switzerland}. The substances were dissolved in 0.9 % NaCl, and the doses used were 100 ng kg^{-1} (40 pmol kg^{-1}) for ET-1 and 5 mg kg^{-1} (8.7 µmol kg^{-1}) for bosentan, which resulted in injection volumes of 270–850 µl (including 200 µl of 0.9 % NaCl to flush the drug through the catheter). Pilot experiments and the experiment with bosentan (see Fig. 4) showed that these volumes of vehicle had no significant effects on the variables measured.

A Leitz Ortholux microscope equipped with an Ultropak water immersion objective (22×) and a digital video camcorder (Sony, DCR-PC7E) were used to observe the vasculature directly in the distal third of the filaments on the second gill arch, as described previously (Nilsson et al., 1995; Sundin and Nilsson, 1998). One filament was studied in each fish examined. Pillar cell cross-sectional area was estimated by assuming that the cells were spherical in shape.

Data treatment

The following calculations were performed: $R_{sys}=P_{DA}/\dot{Q}$, where R_{sys} is systemic resistance; $R_g=(P_{VA}-P_{DA})/\dot{Q}$, where R_g is gill resistance (assuming a small and constant venous overflow in the gill). All data are presented as means ± S.E.M. Kruskal–Wallis non-parametric analysis of variance was used to evaluate statistically significant differences in blood pressure, vascular resistance and heart rate. If a significant difference was found ($P<0.05$), Dunn's multiple comparisons test was used *post-test* to compare all time points with the last pretreatment value. Comparisons of blood oxygen tensions and pillar cell diameters were made using paired Mann–Whitney *U*-tests. The statistical tests were performed using GraphPad Prism 2.0 for Macintosh.

Results

Cardiovascular responses

Injection of ET-1 (100 ng kg^{-1}) into the ventral aorta resulted in several significant changes in the cardiovascular variables measured (Fig. 1). The direction of these changes was the same in all six fish, but most of the variance in the data was the result of an extreme reduction in $\dot{Q}$ (down to 9 % of the pre-injection value within 5 min) in one individual. In the five remaining cod, $\dot{Q}$ had fallen to 84±3 % of the pre-injection level after 5 min, and the corresponding changes in the other variables were as follows: P_{VA} increased from 3.60±0.23 to 4.76±0.27 kPa; P_{DA} and heart rate remained unchanged; R_g increased by 87±13 %; R_{sys} increased by 27±5 %. All these changes were statistically significant.

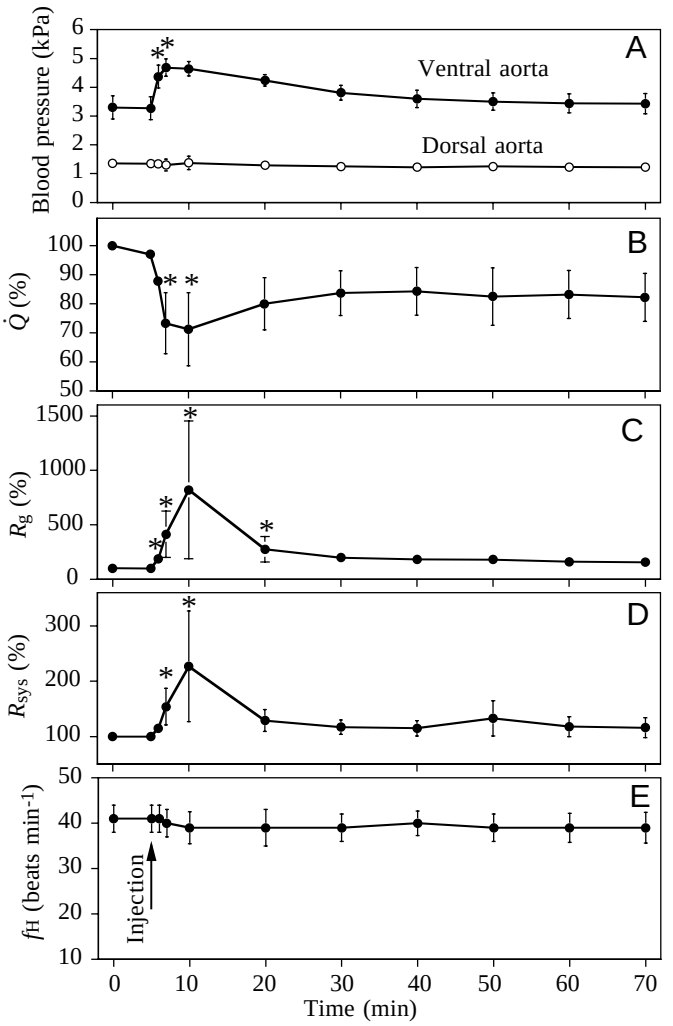

Fig. 1. (A–E) Changes in (A) ventral and dorsal aortic pressure, (B) cardiac output ($\dot{Q}$), (C) gill resistance (R_g), (D) systemic resistance (R_{sys}) and (E) heart rate (f_H) in cod injected with 100 ng kg^{-1} endothelin-1 (ET-1) (into the ventral aorta; marked by an arrow). Values are means ± S.E.M. from six animals. An asterisk indicates a significant difference from the pre-injection value (P<0.05).

The dose of ET-1 chosen was submaximal. In pilot experiments, two cod were injected with 400 ng kg^{-1} ET-1. In one of these, P_{VA} increased from 3.39 to 5.90 kPa, P_{DA} and heart rate remained unchanged, $\dot{Q}$ fell to 67 % of the pre-injection level, R_g increased by 273 % and R_{sys} increased by 41 %. However, in the second fish, 400 ng kg^{-1} ET-1 induced a circulatory collapse resulting in cardiac arrest within 4 min.

Epi-illumination microscopy

The *in vivo* microscopic observations, which were all made on the distal third of filaments on the second gill arch, showed that 100 ng kg^{-1} ET-1 caused a striking increase in the diameter of the pillar cells of the lamellae and a reduction in intralamellar blood flow. The velocity of the erythrocytes passing through the lamellar space (i.e. between the pillar cells) was markedly reduced, and the erythrocytes became trapped between pillar cells. At the same time, the velocity of the blood flowing through the outer marginal channels increased (to such a degree that it was not possible to measure the flow rate). In general, the first signs of these changes were seen 20–40 s after the ET-1 injection. There was no reduction in the diameter of the afferent and efferent filament arteries.

Although this pattern of change was observed in all six cod given 100 ng kg^{-1} ET-1, it was often difficult to make direct measurements of pillar cell diameter after ET-1 injection. This was either because slight movements of the gills made it impossible to follow the same pillar cells or because the outline of the pillar cell was only clearly visible as long as erythrocytes were passing through the lamella (their paths revealing the edges of the pillar cells). The slow-down and cessation of erythrocyte movement in the presence of ET-1, in combination with the increased pillar cell cross-sectional area, made the lamella look almost like a homogeneous mass without any clear borders between the cells.

However, in three cod, the image of the lamellae was at times particularly steady and clear, allowing us to measure the diameters of the pillar cells on frames grabbed from the video tape before and after ET-1 injection. Lamellae from two of these individuals are shown in Fig. 2A–D, and the changes in pillar cell diameter (and estimated cross-sectional area) were as follows. Before ET-1 injection in the first cod (Fig. 2A), the mean diameter of the marked pillar cells (on two lamellae) was 11.9±0.6 μm and their estimated cross-sectional area was 114±11 μm^2. After 30 s (Fig. 2B), the diameter had increased to 15.2±0.5 μm and the cross-sectional area to 182±11 μm^2 (N=14, P=0.0005). Before ET-1 injection in the second cod (Fig. 2C), the mean diameter of the marked pillar cells was 10.5±0.3 μm and the estimated cross-sectional area was 87±5 μm^2. After 240 s (Fig. 2D), the diameter had increased to 15.5±0.6 μm and the cross-sectional area to 192±15 μm^2 (N=12, P=0.002). In these two individuals, the pillar cells could be observed for 30 s and 240 s after the ET-1 injection, respectively, whereupon the borders between pillar cells became too diffuse for measurements to be made. In a third cod, the onset of the effect of ET-1 was particularly fast, the pillar cell diameter (and estimated cross-sectional area) having increased from 10.8±0.6 μm (93±10 μm^2) to 15.4±0.6 μm (188±15 μm^2) (N=6, P=0.03) within 10 s of the ET-1 injection, whereupon pillar cell borders became diffuse. In general, the pillar cells appeared to have regained their normal size after 20–30 min (coinciding with the recovery of P_{VA} and $\dot{Q}$, see Fig. 1A,B).

Blood oxygen tensions

The P_{O_2} in the ventral and dorsal aorta was measured 5–8 min after the ET-1 injection (100 ng kg^{-1}) in five of the cod (Fig. 3). In the ventral aorta, P_{O_2} fell significantly (P=0.03) from 3.2±0.2 to 2.0±0.3 kPa. In contrast, there was no significant reduction in the dorsal aortic P_{O_2} (11.0±1.5 kPa before and 10.0±1.2 kPa after ET-1 injection). The individual showing an extreme fall in $\dot{Q}$ after ET-1 injection (see above)

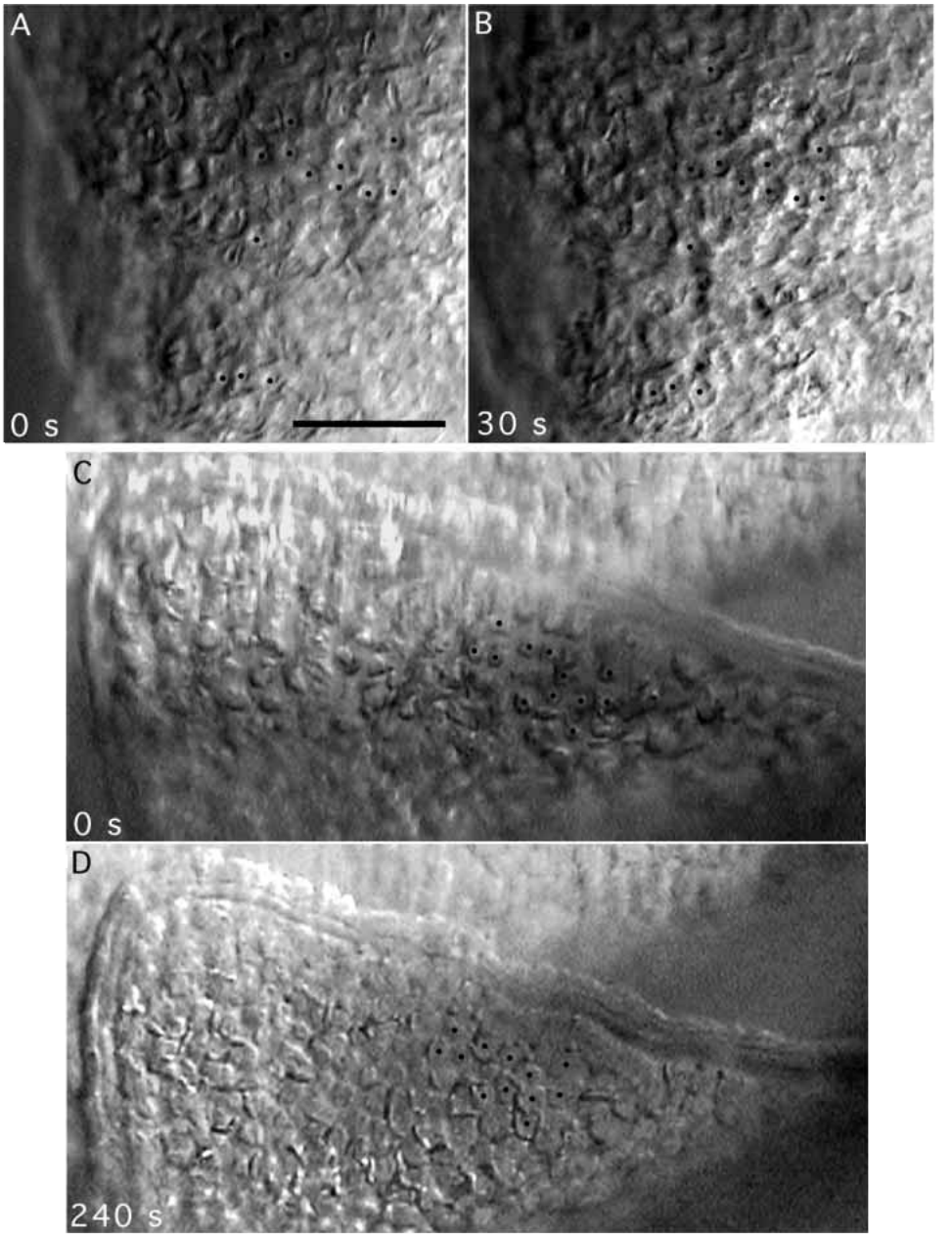

Fig. 2. (A–D) Increased pillar cell diameter, consistent with pillar cell contraction, as shown by micrographs of the efferent portion of the gill lamellae of two cod (A,B for cod 1; C,D for cod 2) before and after an injection of endothelin-1 (ET-1) (100 ng kg^{-1}). In both fish, a number of pillar cells have been marked by black dots. Note that the vascular space between the pillar cells becomes reduced after ET-1 injection as a result of the apparent pillar cell contraction. In the second individual (D) particularly, this causes erythrocytes (seen as elongated objects) to be trapped between the pillar cells. The diffuse bands running horizontally through the middle and bottom of A and B are the outer marginal channels. The rate of blood flow in the outer marginal channels increased markedly after ET-1 injection so that some erythrocytes can be seen in the marginal channels as sharp elongated objects in A but not in B. In the second individual (C,D), the outer marginal channel is clearly seen running along the upper and left edges of the lamella. The illustrations are digital video frames imported to Adobe Photoshop 3.0 on a Macintosh PowerPC equipped with a digital video frame grabber card (Sony DVBK-2000E). Scale bar, 100 μm.

also underwent a larger reduction in aortic P_{O_2}: from 3.4 to 1.3 kPa in the ventral aorta and from 13.0 to 8.4 kPa in the dorsal aorta.

ET receptor antagonism by bosentan

At a dose of 5 mg kg^{-1}, bosentan was found to block completely the ability of ET-1 to alter the cardiovascular variables measured (Fig. 4) and the lamellar blood flow patterns. This blockage even occurred when ET-1 was given at a dose of 400 ng kg^{-1}. As mentioned above, this high ET-1 dose had profound circulatory effects and induced a circulatory collapse in one fish. Bosentan (5 mg kg^{-1}) by itself had no significant effects on the cardiovascular variables (Fig. 4) and no observable effects on lamellar blood flow. However, it should be mentioned that, in a pilot experiment, one cod was injected with a higher dose of bosentan (15 mg kg^{-1}). This individual showed a marked reduction in lamellar blood flow (as seen through the microscope) and displayed cardiovascular changes in the same direction as those seen after ET-1 injection, suggesting that bosentan may function as an ET-1 agonist at doses higher than 5 mg kg^{-1}.

Discussion

Our results show that injection of picomolar amounts of ET-1 into the ventral aorta of cod causes a significant increase in the vascular resistance of the gills. Simultaneously, there was an increase in the pillar cell diameter and an intralamellar shift of blood from the lamellar sheet towards the outer marginal channels, where blood flow increased markedly. The filament

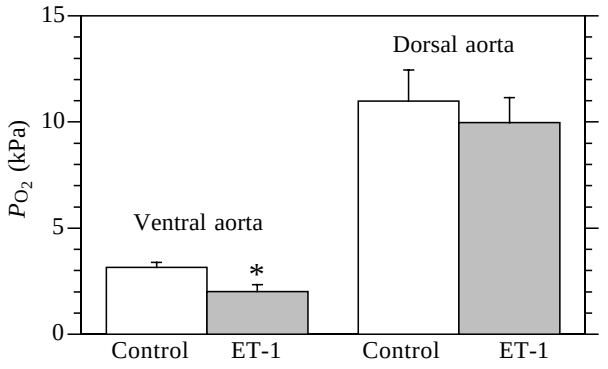

Fig. 3. Oxygen tension (P_{O_2}) in the ventral and dorsal aorta measured before (control) and 5–8 min after an injection of endothelin-1 (ET-1) (100 ng kg^{-1}). Values are means ± S.E.M. from five cod. An asterisk indicates a significant difference from the pre-injection value (P<0.05) (paired Mann–Whitney U-test).

arteries in the portion of the filaments we observed did not constrict. The only explanation we can find for these observations is that ET-1 induces a contraction of pillar cells, causing a decrease in the vascular space between them. In support of this conclusion is the observation that pillar cells are the only cells in the lamellae where putative contractile elements (myosin-like microfilaments) have been observed (Smith and Chamley-Campbell, 1981; Newstead, 1967; Bettex-Galland, 1973).

The ability of ET-1 to contract pillar cells and to redistribute lamellar blood flow may be widespread among teleost fishes. In a previous study on the branchial effects of ET-1 in rainbow trout (Sundin and Nilsson, 1998), an increase in pillar cell diameter was apparent after ET-1 injection, the blood flow often coming to a complete stop in the lamellar sheet, while outer marginal channel blood flow was increased. However, these observations were not backed up by measurements of $\dot{Q}$ and dorsal aortic pressure, so changes in vascular resistance could not be calculated.

A specific ET-1-induced contraction of pillar cells corresponds well with the limited distribution of ET-1 binding sites in rainbow trout gill filaments; they are found only in the lamellae (Lodhi et al., 1995). Such a limited branchial distribution of ET receptors explains the absence of any noticeable vasoconstriction of lamellar arterioles, filamental arteries or outer marginal channels after ET-1 injection. It is also worth pointing out that experiments on *in vitro* preparations from cod, salmon (*Salmo salar*) and rainbow trout show that rings cut from the ventral aorta, which could almost be regarded as part of the branchial vasculature, are much less sensitive to ET-1 than some other vascular preparations, including the trunk, dorsal aorta and coeliaco-mesenteric artery (Olson et al., 1991; Sverdrup et al., 1994). There is also an interesting parallel between mammals and fish. The mammalian alveoli, which correspond functionally to the gill lamellae, contain endothelin receptors that appear to be responsible for smooth muscle contraction in this tissue (Goldie et al., 1996a,b).

Interestingly, in a study of several teleost species, ET-immunoreactivity was detected in endothelial cells of gill arteries and neuroendocrine cells in the gill filaments (Zaccone et al., 1996). It is possible that these cells are sources of endogenous ET acting downstream on the lamellae.

We have previously used *in vivo* microscopy to study the branchial microcirculatory effects of acetylcholine, adenosine and serotonin, all potent gill vasoconstrictors. These three substances were all found to act at the level of the filamental arteries and lamellar arterioles (Sundin et al., 1995; Sundin and Nilsson, 1996, 1997), and we never observed signs of pillar cell contraction. Thus, the effects of ET-1 on the gill vasculature appear to be unique among the substances that have been studied so far.

We find it unlikely that the reduction in intralamellar flow and the increase in pillar cell diameter after ET-1 injection are caused by a passive lamellar collapse, due to a reduction in intralamellar pressure, rather than to pillar cell contraction. The intralamellar blood pressure should be at least as high as P_{DA}, which did not fall after ET-1 injection. If intralamellar blood pressure were lower than P_{DA}, we would expect to see blood flowing in the reverse direction, i.e. from the efferent filament artery into the lamellae. This was never observed. In fact, the flow rate in the outer marginal channel increased markedly in response to ET-1 injection, which indicates an increased intralamellar pressure.

However, since only a small proportion of the filaments were under observation, we cannot conclude that all lamellae

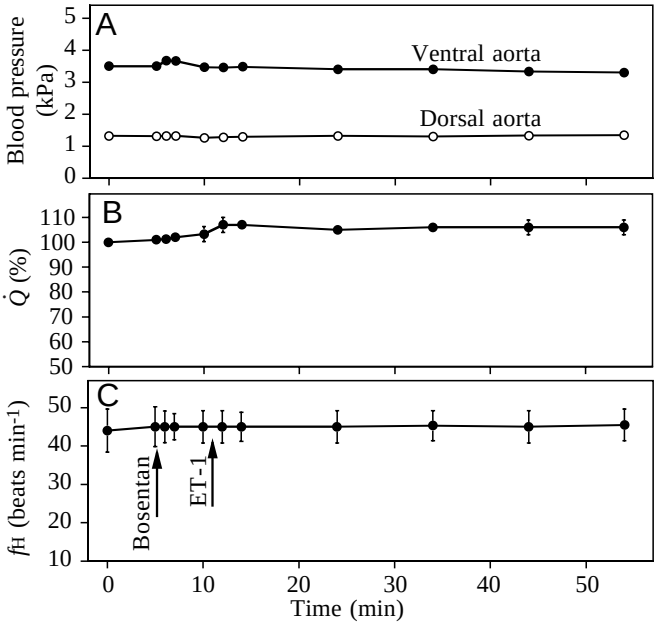

Fig. 4. (A–C) Effects of an injection of bosentan (5 mg kg^{-1}) and a subsequent injection of endothelin-1 (ET-1) (400 ng kg^{-1}) on (A) ventral and dorsal aortic pressure, (B) cardiac output ($\dot{Q}$) and (C) heart rate (f_H) in cod. The injections (marked by arrows) were made in the ventral aorta. Values are means ± S.E.M. from four cod. No statistically significant effects of bosentan or ET-1 were found (Kruskal–Wallis non-parametric analysis of variance).

are constricted or equally constricted. The possibility remains that the lamellae we observed (all localised in the distal third of the filaments) are preferentially shut down by ET-1, and that ET-1 is involved in regulating lamellar recruitment/derecruitment.

ET-1 had no effect on heart rate in cod. Similarly, in rainbow trout, Olson et al. (1991) found that ET-1 infusion was without effect on heart rate. The same authors also observed that ET-1 did not affect the rate and maximum power output of the *in situ* perfused trout heart and that higher concentrations of ET-1 were needed to contract trout coronary arteries compared with other parts of the trout vasculature.

The increase in R_{sys} seen after ET-1 treatment in cod is indicative of a widespread vasoconstrictory effect of ET-1 in this species. This is in agreement with previous observations. ET-1 has been shown to constrict dorsal aortic rings of cod and salmon (Sverdrup et al., 1994), rings from the dorsal aorta and coeliaco-mesenteric artery of trout (Olson et al., 1991) and the mesenteric artery and posterior cardinal vein of a catfish (*Amiurus melas*) (Poder et al., 1991). In trout, ET-1 has also been found to increase the vascular resistance of perfused trunk preparations (Olson et al., 1991; Brown and Amer, 1997).

A reduced flow of erythrocytes through the lamellar sheet could be expected to have profound effects on gas exchange (Richards and Fromm, 1969; Morgan and Tovell, 1973). Surprisingly, we found that the P_{O_2} in the dorsal aorta was not significantly reduced after ET-1 injection, although the lamellar flow was clearly reduced. This probably relates to the concomitant reduction in $\dot{Q}$: the prolonged passage time for the erythrocytes through the gills still allowed the blood to be oxygenated. However, the lowered $\dot{Q}$ also means that less oxygen is transported to the body. To compensate for this, the body would have to strip the blood of more of its oxygen content. Indeed, in the blood returning to the gills (i.e. the ventral aortic blood), there was a significant reduction in P_{O_2} from 3.2 to 2.0 kPa. This reduction in P_{O_2} probably represents a considerable reduction in the amount of oxygen present in the blood. According to the haemoglobin oxygen-dissociation curve for cod described by Fritsche (1993), a reduction in ventral aortic P_{O_2} from 3.2 to 2.0 kPa would correspond to a reduction in the haemoglobin oxygenation level from 50 to 5 % if the CO_2 level were simultaneously increased (which should be the case). The same dissociation curve also shows that the haemoglobin is approximately 90 % saturated with oxygen at the dorsal aortic P_{O_2} measured in our cod (10–11 kPa).

It is of interest to note that the effect of ET-1 on the gill vasculature had a faster onset in cod than in trout, where a maximal effect was seen after 5 min (Sundin and Nilsson, 1998) compared with 1–2 min or less in the cod. This might relate to differences in the rate at which intracellular mechanisms mediate the effects of ET-1.

Of significance for future studies of the physiological role of ET-1 in teleosts was our finding that bosentan, at a dose of 5 mg kg^{-1}, completely inhibited the effect of ET-1, even when ET-1 was administered at a dose four times higher than that used regularly in this study. This is the first time an ET-1 receptor antagonist has been shown to inhibit the vascular effects of ET-1 in fish. In itself, this dose of bosentan had no significant haemodynamic effects, indicating that there is no tonic influence of endothelins on the vasculature of anaesthetised cod. Interestingly, experiments with bosentan have shown that this is also the case in anaesthetised dogs (Teerlink et al., 1995).

Bosentan is a non-peptide ET antagonist that blocks both subtypes of ET receptor (ET_A and ET_B) in mammals. It appears to be highly specific for ET receptors, since it has been shown to be without effect on the receptor binding of 40 other peptides, prostaglandins, ions and neurotransmitters (Clozel et al., 1994).

The pharmacological nature of the ET receptor(s) in fish is uncertain. Lodhi et al. (1995) suggested that the ET receptor they detected in trout gill lamellae was a novel fish-type ET_A receptor, denoted ET-AF. They found that BQ-123, a specific antagonist of the mammalian-type ET_A receptor, and BQ-3020, a specific agonist of the mammalian-type ET_B receptor, both had a very low affinity for the trout ET-1 receptor. Similarly, Evans et al. (1996) found that BQ-123 was unable to block ET-1-induced constriction of ventral aortic rings from spiny dogfish (*Squalus acanthias*). However, they found that sarafotoxin S6c, a specific agonist of the mammalian ET_B receptor, also constricted the aortic rings, suggesting that the ET receptor involved was of the ET_B type rather than the ET_A type.

The most important aspect of the present study may be that it shows that fish have the ability to redistribute lamellar blood flow from the lamellar sheet to the outer marginal channel through local vasoconstriction (pillar cell contraction). Such a mechanism would allow fishes to optimise their functional respiratory surface area to their instantaneous requirement for oxygen.

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