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EFFECTS OF EXERCISE, HYPOXIA AND FEEDING ON THE GASTROINTESTINAL BLOOD FLOW IN THE ATLANTIC COD *GADUS MORHUA*

BY MICHAEL AXELSSON AND REGINA FRITSCHE*

Comparative Neuroscience Unit, Department of Zoophysiology, University of Göteborg, PO Box 250 59, S-400 31 Göteborg, Sweden

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Summary

Cardiac output, ventral and dorsal aortic blood pressure, heart rate, and coeliac and mesenteric artery blood flow were recorded simultaneously in the Atlantic cod, *Gadus morhua* L., at rest, during exercise, during hypoxia and after feeding.

In the resting unfed animals, coeliac artery blood flow was 4.1 ± 0.8 ml $min^{-1} kg^{-1}$ and mesenteric artery blood flow was 3.5 ± 1.1 ml $min^{-1} kg^{-1}$ (mean± S.E.M., $N=10$); together, these flows represent approximately 40 % of the cardiac output.

Exercise or exposure to hypoxia resulted in increased visceral vascular resistance, leading to reductions in the coeliac and mesenteric artery blood flows.

Coeliac and mesenteric blood flows were increased 24 h after feeding and the coeliac and systemic vascular resistances decreased in comparison with the prefeeding values. Phentolamine did not affect the gastrointestinal artery blood flow, but produced a significant decrease in the mesenteric and systemic vascular resistance.

Treatment with bretylium and phentolamine revealed differences between the coeliac and the mesenteric vasculature regarding the control mechanisms during hypoxia and during exercise and feeding. During hypoxia, an adrenergic control of the gastrointestinal vasculature with both nervous and humoral components was found, whereas during exercise and after feeding an additional non-adrenergic mechanism controlling gut blood flow was demonstrated.

Introduction

The general mechanisms involved in the control of the heart and vasculature in fish *in vivo* are reasonably well understood (for references, see Holeton and Randall, 1967; Daxboeck and Holeton, 1978; Randall and Daxboeck, 1982; Axelsson and Nilsson, 1986; Gehrke and Fielder, 1988; Fritsche and Nilsson, 1990), but there are still relatively few reports on the regional distribution of the

* To whom reprint requests should be sent.

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cardiac output or the control mechanisms involved during exercise and hypoxia (Laurent *et al.* 1983; Nilsson, 1983; Farrell, 1984).

Regulation of the total systemic vascular resistance in the Atlantic cod (*Gadus morhua*) and rainbow trout (*Oncorhynchus mykiss* [=*Salmo gairdneri*]) is mediated *via* tonically active adrenergic nerves, with little or no contribution from circulating catecholamines (Smith, 1978; Smith *et al.* 1985; Axelsson and Nilsson, 1986). During exercise, cardiac output increases as a result of an increase in both heart rate and stroke volume, while the systemic resistance either decreases (rainbow trout) or remains virtually unchanged (Atlantic cod) (Kiceniuk and Jones, 1977; Daxboeck, 1981; Randall and Daxboeck, 1982; Axelsson and Nilsson, 1986). Hypoxic exposure, however, induces an elevation of the total systemic resistance in the Atlantic cod without any change in cardiac output (Fritsche and Nilsson, 1989). The change in systemic vascular resistance during exercise and hypoxia is mediated *via* adrenergic nerves and, during hypoxia, also to a certain extent *via* circulating catecholamines (Kiceniuk and Jones, 1977; Randall and Daxboeck, 1982; Axelsson and Nilsson, 1986; Fritsche and Nilsson, 1990).

The systemic vascular circuit consists of at least two distinct parts: the visceral and the somatic circulation. The reactions of the visceral circulation during exercise and hypoxia, or after feeding, have not been studied extensively in fish. There are some indications of a redistribution of cardiac output away from the gut vasculature during exercise in the rainbow trout (Daxboeck, 1981). Changes in the gastrointestinal vascular resistance may also affect the systemic blood pressure in fish, as suggested in the study by Axelsson *et al.* (1989) on the sea raven (*Hemitripterus americanus*).

In mammals, the diffuse adrenergic sympathetic nervous discharge seen during exercise increases the vascular resistance in the gastrointestinal circulation, while the local metabolically induced vasodilatation in the working muscles is unaffected by this discharge (Orwell *et al.* 1964; Donald, 1980). Therefore, the fraction of the cardiac output reaching the gut is reduced during exercise (Rushmer *et al.* 1961; Vatner *et al.* 1971).

Feeding has been shown to cause an increased gastrointestinal blood flow in mammals, but whether this is due to an increased cardiac output or a selective increase in visceral blood flow is not clear (for a discussion, see Fara, 1984). In the sea raven (*Hemitripterus americanus*) it was shown that the coeliac artery blood flow increased as a result of feeding and that visceral vascular resistance was, at least in part, controlled by an α-adrenoceptor-mediated mechanism (Axelsson *et al.* 1989).

The control of the visceral blood flow and resistance, at least in mammals, is mediated largely by noradrenergic nerves. However, the rapidly increasing numbers of reported non-adrenergic, non-cholinergic (NANC) neurotransmitters and hormones of the gut raise the question of a control of the gut circulation by non-adrenergic agents, e.g. neuropeptides (Nilsson and Holmgren, 1989).

The present study has the following major objectives: first, to measure the gastrointestinal blood flow in resting, unrestrained fish and to determine the ro

of circulating catecholamines and adrenergic nerves in the control of gut blood flow; and second, to study the effects of exercise, hypoxia and feeding on visceral blood flow.

Materials and methods

The study was carried out on Atlantic cod, *Gadus morhua* L., of both sexes, with a body mass of 550–1050 g and a body length of 38–45 cm. The fish were kept in well-aerated, recirculating sea water at 10–11° C. The animals were acclimated to the departmental seawater system for 1 week before surgery and used within 2 weeks of capture. The fish were never fed in captivity. The exercise and feeding experiments were performed in August–November 1989 and the hypoxia experiments in May–June 1990.

Surgical and preparative procedures

The fish were anaesthetized in MS 222 (tricaine methane sulphonate; 100 mg l^{-1}, Sigma), until breathing movements ceased, and transferred to an operating table, where aerated sea water containing the anaesthetic (50 mg l^{-1}) was passed over the gills throughout the surgery. A cannula (PE 50) filled with heparinized (100 i.u. ml^{-1}) 0.9 % NaCl was inserted into the afferent branchial artery of the third gill arch for measurement of ventral aortic blood pressure (P_{VA}) and heart rate (f_H), and for taking blood samples. A second cannula was occlusively inserted into the efferent branchial artery of the same gill arch for measurement of dorsal aortic pressure (P_{DA}) and injection of adrenergic antagonists. Both cannulae were secured with skin sutures.

In order to measure cardiac output ($\dot{Q}$=ventral aortic blood flow), the ventral aorta was exposed *via* an incision immediately anterior to the base of the pectoral fins. The ventral aorta was carefully dissected free from the surrounding tissue without rupturing the pericardium, and a cuff-type Doppler flow probe (2.5–3.0 mm i.d., single crystal, P. Pohl International Inc.) was placed around the ventral aorta.

In the cod, the gastrointestinal tract is supplied with blood *via* the coeliac and the mesenteric arteries. These arteries branch off the right suprabranchial artery as the coeliaco-mesenteric artery, which divides into the two main visceral arteries: the coeliac artery supplies the stomach–liver region including the pyloric caeca, while the mesenteric artery supplies part of the stomach and the intestine.

To record coeliac and mesenteric artery blood flow (q_{CoA}, q_{MeA}) the animal was placed on its right side and an incision was made between the pectoral fin and the pelvic fin. The coeliac and mesenteric arteries were freed from the surrounding tissue, taking great care not to damage the nerves running along each of these vessels. Both vessels were tightly fitted with a cuff-type Doppler flow probe (1.3–1.6 mm i.d., single crystal, P. Pohl International Inc.). The leads from the flow probes were tunnelled to the outside just behind the right pectoral fin, and

each was secured with two skin sutures. The Doppler flow probes were connected to a Doppler flow meter (Iowa University).

The cannulae were attached to Honeywell pressure transducers (model 156PC06GW2). Calibration of the transducers was made electrically *via* specially constructed preamplifiers, and the electrical calibration was occasionally checked using a static water column. The pressure and flow signals were suitably amplified and displayed on a Grass Polygraph recorder system (model 7D). f_H was derived from the phasic blood pressure (P_{VA}) signals *via* a Grass 7P44 tachograph, and expressed as beats min^{-1}. $\dot{Q}$, q_{CoA}, q_{MeA} and stroke volume (V_S) are expressed in kHz Doppler shift, or percentage changes from control values.

After surgery, the animals were transferred to the experimental chambers and allowed to recover for at least 24 h before any experiments were conducted. During this time the effects of anaesthesia and handling wore off and the cardiovascular parameters reached steady levels (Smith *et al.* 1985).

Experimental protocol

The experimental protocol used is similar to that used in earlier studies of cod cardiovascular physiology (Smith *et al.* 1985; Axelsson and Nilsson, 1986; Fritsche and Nilsson, 1990). Three different groups of animals were used to study the effects of exercise, hypoxia and feeding on the blood supply to the gastrointestinal canal.

Sequential injection of drugs was used to abolish the influence of the adrenergic nerves and circulating catecholamines. This procedure allows comparisons of the different variables of each individual before and after a certain treatment. Bretylium tosylate (a gift from the Wellcome Foundation) was used to abolish the effects of adrenergic nerves on the circulatory system (Smith *et al.* 1985).

Since the primary objective of this study was to determine gut blood flow control, i.e. vasomotor events in the visceral vasculature, further elucidation of, for example, cardiac function using β-adrenoceptor antagonists was not made (see, however, Fritsche and Nilsson, 1990).

Phentolamine methanesulphonate (Ciba-Geigy) was used to abolish the remaining α-adrenoceptor-mediated adrenergic vasomotor control (i.e. control *via* circulating catecholamines). The drugs were dissolved in 0.9 % NaCl. Injections were made through the dorsal aortic cannula and the injected volume was 1 ml kg^{-1}. The selectivity of the drugs in the doses used has been established in studies of both cod (Smith *et al.* 1985; Axelsson and Nilsson, 1986; Fritsche and Nilsson, 1990) and toad (Wahlqvist and Campbell, 1988).

Exercise

After surgery, the animals were transferred to a water channel (see Axelsson and Nilsson, 1986) and left to recover for at least 24 h. The water in the channel was steadily replaced (2 l min^{-1}) from the departmental seawater system. The temperature was kept between 10 and 11 °C in all experiments.

The experiment was started by recording resting variables in untreated anima

(*untreated rest*) of P_{VA}, P_{DA}, $\dot{Q}$, q_{MeA}, q_{CoA} and f_H, and a blood sample (0.3 ml) was taken for later catecholamine analysis. The water flow in the swim channel was then started and the speed adjusted to 2/3 body lengths s^{-1} (Axelsson and Nilsson, 1986). The exercise period lasted 10 min and during that time the cardiovascular variables were recorded (*untreated exercise*). At the end of this period another blood sample was taken for catecholamine analysis.

After the first experiment, the fish was slowly injected (over 10–20 min) with bretylium tosylate (10 mg kg^{-1}) *via* the efferent branchial artery. The fish was left to recover for another 24 h, during which time the side-effects of bretylium wore off, leaving a blockade of the adrenergic nerves only (Smith *et al.* 1985; Axelsson and Nilsson, 1986; Axelsson, 1988). At this point the resting variables (*bretylium rest*) were again recorded and another exercise period was then performed, and the cardiovascular variables (*bretylium exercise*) were recorded. A blood sample for catecholamine analysis was also taken at the end of this exercise period.

Phentolamine methanesulphonate (2 mg kg^{-1}) was then injected into the bretylium-treated fish. Four hours later, when the recorded variables were stable (*phentolamine rest*), the fish was subjected to a final exercise bout during which recordings of the variables were made (*phentolamine exercise*), and a final blood sample for catecholamine analysis was taken at the end of the exercise period.

Hypoxia

The experiments were conducted using the water channel described by Fritsche and Nilsson (1989). The water in the channel was replaced at a rate of approximately 1 l min^{-1} from the departmental water system at a temperature of 10–11 °C. Oxygen tension in the water channel was continuously recorded by an oxygen electrode placed in front of the fish and connected to a Radiometer PHM71 and Grass polygraph recorder system model 7D.

Control values of P_{VA}, P_{DA}, f_H, $\dot{Q}$, q_{MeA} and q_{CoA} were recorded at the beginning of each experiment (*untreated normoxia*). During that time a blood sample (0.3 ml) was also taken for later catecholamine analysis. Hypoxic water was prepared by bubbling N_2 through a barrel of water. The fish was made hypoxic by switching two three-way stopcocks so that water entered the experimental box from the barrel. The hypoxic period lasted 8 min, during which time the cardiovascular variables were recorded (*untreated hypoxia*). The oxygen tension in the water was rapidly reduced (within 1 min) to a constant level of 4.0–5.3 kPa ($=P_{w_{O_2}}$=30–40 mmHg). At the end of the hypoxic period another blood sample (0.3 ml) was taken for later analysis of catecholamines. The hypoxic period ended by switching to normoxic water.

After the first experiment, the fish was slowly injected (over 10–20 min) with bretylium tosylate (10 mg kg^{-1}) *via* the efferent branchial artery. After 24 h of recovery from the side-effects of bretylium, the cardiovascular variables were again recorded (*bretylium normoxia*), and then another period of exposure to hypoxia was performed and the cardiovascular variables (*bretylium hypoxia*) were recorded.

Phentolamine methanesulphonate ($2\,mg\,kg^{-1}$) was then injected into the bretylium-treated fish and 4 h later the variables were recorded (*phentolamine normoxia*), and then the fish was subjected to a final period of hypoxia (*phentolamine hypoxia*).

Feeding

After surgery, the animals were transferred to the aquarium in which the feeding experiments were performed. The water in the aquarium was continuously replaced ($2\,l\,min^{-1}$) from the departmental seawater system, and the temperature was kept at 10–11° C during the experiments.

After the recovery period, control values of P_{VA}, P_{DA}, $\dot{Q}$, q_{MeA}, q_{CoA} and f_H were recorded (*prefeeding*). The fish were then lightly anaesthetized by adding MS 222 ($100\,mg\,l^{-1}$) to the water. When the animal had lost the righting reflex, approximately $25–35\,g\,kg^{-1}$ body mass of rainbow trout (*Oncorhynchus mykiss*) was introduced into the stomach of the cod using a pair of forceps. The water in the aquarium was changed and the fish regained the righting reflex within a few minutes.

Twenty-four hours after feeding, the variables were again recorded (*postfeeding*) and the animals were then injected with phentolamine ($2\,mg\,kg^{-1}$). Four hours after the phentolamine injection, final recordings of P_{VA}, P_{DA}, $\dot{Q}$, q_{MeA}, q_{CoA} and f_H were made (*phentolamine postfeeding*).

Calibration of the Doppler flow probes

In 10 animals, an *in situ* calibration of the mesenteric and coeliac artery flow probes was performed at the end of the experiment. The animals were killed and the flow probes were calibrated *in situ* after securing an inflow cannula in the right suprabranchial artery and outflow cannulae posterior to the flow probes in both the coeliac and the mesenteric artery. To ensure structural similarity of the vessel, i.e. a 'tight fit' of the flow probe also during calibration, the outflow cannula was raised to about 15–20 cm, which produced the necessary counter-pressure. Heparinized cod blood (Hct=5–8 %), was used to calibrate the probes. The data were then analyzed by linear regression analysis. The flow velocities measured with the Doppler technique showed linear correlation ($r>0.98\pm0.01$; $N=10$) with volume flow; the technique therefore provides an adequate measure of flow rate.

Analysis of catecholamines

Plasma levels of the catecholamines noradrenaline and adrenaline were measured using high performance liquid chromatography (HPLC) with electrochemical detection, as described by Hallman *et al.* (1978). The blood samples were immediately mixed with 20 μl of a solution containing glutathione ($0.2\,mol\,l^{-1}$) and EGTA ($0.2\,mol^{-1}$) and centrifuged to remove the blood cells. The plasma samples were kept frozen at −80°C for no more than 4 weeks before being processed. The extraction and analysis were performed as described by Fritsche and Nilsson (1990).

Data acquisition, calculations and statistics

In addition to the Grass polygraph recordings, a data-acquisition software package (AD/DATA;P. Thorén, University of Göteborg) was used and all data were fed into an IBM PPC computer. The sampling frequency was set to 2 samples s^{-1} and on-line mean value calculations over 5-s periods were performed. Data from individual experiments were superimposed and presented in graphs as means± S.E.M., where each point represents a mean value of six samples (=30 s).

The Wilcoxon's sign rank test for paired samples (two-tailed) was used to determine the statistical significance of the observed effects of exercise, hypoxia and feeding in combination with the different drug treatments. The level of significance was set to $P \leq 0.05$ in all experiments and statistically significant changes are indicated by asterisks and triangles in the figures.

For the statistical tests, a 30-s resting or normoxic period (*untreated rest* and *untreated normoxia*) was compared with a 30-s period at the end of the exercise and hypoxic period (*untreated exercise* and *untreated hypoxia*). After drug treatment the same comparisons were made; *bretylium* rest and *bretylium normoxia* were compared with *bretylium exercise* and *bretylium hypoxia*, respectively, and *phentolamine rest* and *phentolamine normoxia* were compared with *phentolamine exercise* and *phentolamine hypoxia*, respectively.

To compare the exercise and hypoxic variables before and after each drug treatment the following comparisons were also made: *untreated exercise* with *bretylium exercise*; *bretylium exercise* with *phentolamine exercise*; *untreated hypoxia* with *bretylium hypoxia*; *bretylium hypoxia* with *phentolamine hypoxia*. In the feeding experiments, a 3-min period, 24 h after surgery (*prefeeding*) was compared with a 3-min period 24 h after feeding (*postfeeding*) and, finally, a 3-min period 4 h after the phentolamine injection (*phentolamine postfeeding*) was compared with the 24 h postfeeding (*postfeeding*) period. When variables were used in more than one comparison in the statistical evaluation, a sequentially rejective Bonferroni test (Holm, 1979) was used to eliminate, as far as possible, the risk of discarding any true null hypothesis.

The systemic (R_s), the mesenteric (R_{MeA}) and the coeliac (R_{CoA}) vascular resistances were calculated as the pressure drop across the vascular bed divided by the blood flow through the same vascular circuit. For these calculations, three assumptions were made: (1) P_{DA} equals the blood pressure in the mesenteric and coeliac artery; (2) central venous blood pressure is zero and does not change significantly during the experiment (Kiceniuk and Jones, 1977); (3) there is no change in the blood viscosity during the experiment. For further discussion about vascular resistance calculations, see Greenway (1982).

Results

The recorded values of P_{VA}, P_{DA}, f_H compare well with previously recorded values of these variables in the cod (Smith *et al.* 1985; Axelsson and Nilsson, 1986; Axelsson, 1988; Fritsche and Nilsson, 1989, 1990). The mesenteric and coeliac

artery blood flow probes were calibrated in 10 animals, and the mean control blood flow in the coeliac artery was found to be $4.1\pm0.8\,ml\,min^{-1}kg^{-1}$; the corresponding blood flow in the mesenteric artery was $3.5\pm1.1\,ml\,min^{-1}kg^{-1}$.

Exercise

During exercise, in untreated animals, there were statistically significant increases in P_{VA}, P_{DA}, f_H, $\dot{Q}$, R_{MeA} and R_{CoA}. The increase in $\dot{Q}$ (50%) was mediated by a significant increase in f_H (16%), while the increase in V_S was not significant. The gastrointestinal blood flow was significantly reduced: q_{CoA} by 29% and q_{MeA} by 36%, as a result of significant increases in the visceral vascular resistances by 75% (R_{CoA}) and 101% (R_{MeA}). The exercise also induced a significant reduction of the total systemic vascular resistance by 22% (Figs 1 and 2).

Injection of bretylium caused a significant reduction in f_H (14%) and an increase in q_{MeA} (32%) in the resting cod (*bretylium rest*). During exercise in the bretylium-treated animals (*bretylium exercise*) there were significant increases in f_H, $\dot{Q}$, R_{CoA} (56%) and R_{MeA} (33%), while significant decreases in q_{CoA} (40%), q_{MeA} (33%) and R_S (41%) were found. Compared with that in the untreated fish (*untreated exercise*), the exercise-induced reduction in q_{MeA} was significantly smaller (−36% and −33%, respectively).

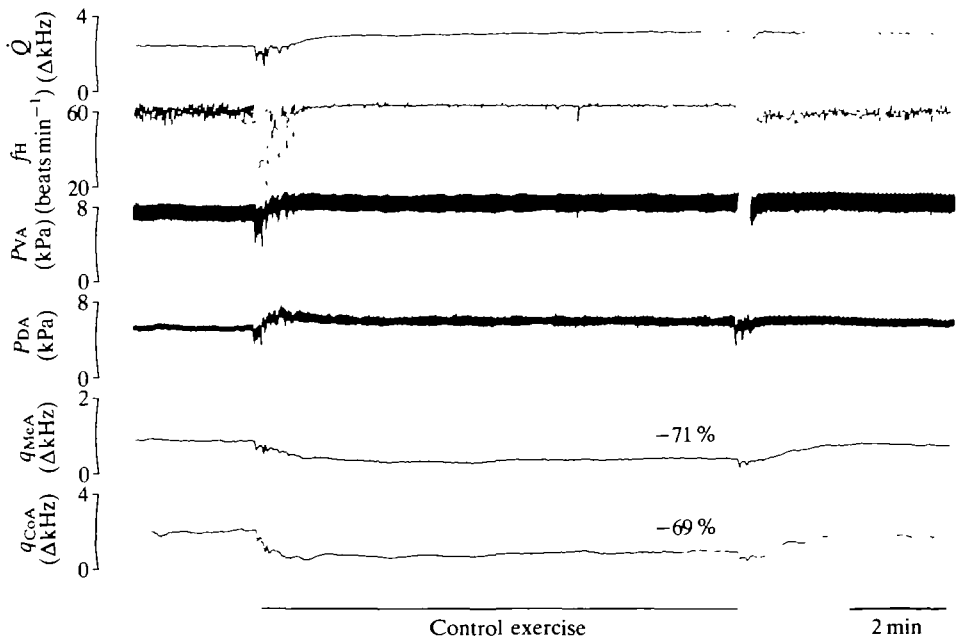

Fig. 1. Simultaneously recorded $\dot{Q}$, f_H, P_{VA}, P_{DA}, q_{MeA} and q_{CoA} in a cod before, during and after an exercise period. The percentage changes in q_{MeA} and q_{CoA} in this particular animal are indicated in the figure. Abbreviations are explained in the text.

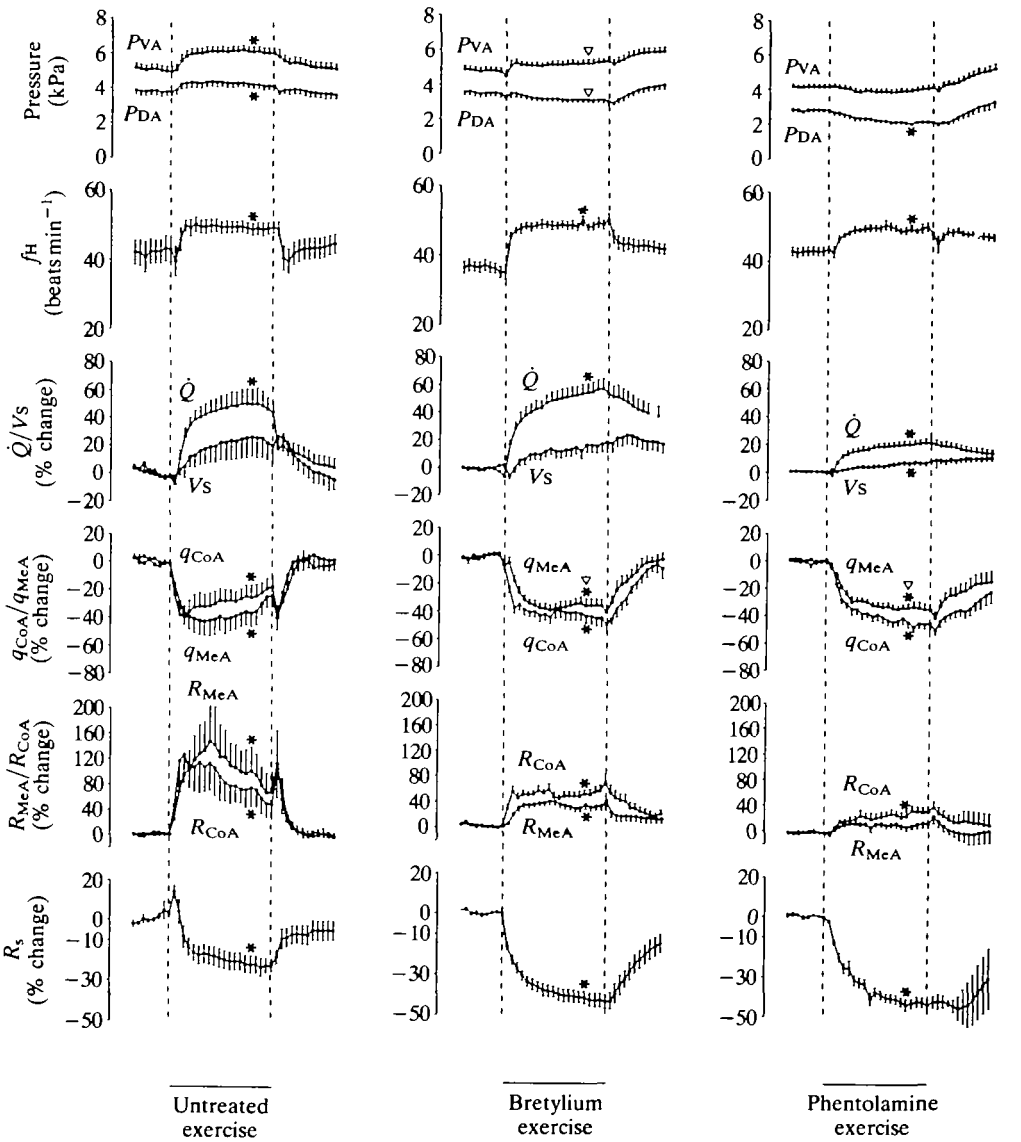

Fig. 2. A summary of cardiovascular responses to 10 min of exercise in the Atlantic cod, in untreated animals (untreated exercise) in the same animals after treatment with bretylium (bretylium exercise) and after additional phentolamine treatment (phentolamine exercise), N=8–9. Mean values±S.E.M. are presented. Asterisks indicate statistically significant ($P \leq 0.05$) differences compared with rest values. Triangles indicate statistically significant ($P \leq 0.05$) differences compared with the value prior to treatment (bretylium and the subsequent phentolamine treatment). Abbreviations are explained in the text.

Following the phentolamine injection there were significant reductions in the resting values (*phentolamine rest*) of P_{DA} and P_{VA}, while f_H increased by 17 %, $\dot{Q}$ by 21 % and q_{MeA} by 19 % compared with values in bretylium-treated fish. During the final exercise bout (*phentolamine exercise*), there were significant decreases in P_{DA} (12 %), q_{CoA} (44 %), q_{MeA} (34 %) and R_S (44 %). At the same time, there were significant increases in f_H (14 %), $\dot{Q}$ (35 %), V_S (18 %) and R_{CoA} (38 %). There were no significant increases in either adrenaline or noradrenaline levels during exercise, or in the exercising fish after bretylium injection. Only after the combined bretylium+phentolamine treatment did the plasma concentration of adrenaline increase (see Fig. 5).

Hypoxia

During hypoxia (*untreated hypoxia*), there were statistically significant increases in P_{VA}, P_{DA}, $\dot{Q}$, R_{MeA} and R_{CoA}. The increase in $\dot{Q}$ (32 %) was mediated by an increase in V_S (29 %). The gastrointestinal blood flow was significantly reduced: q_{CoA} by 42 % and q_{MeA} by 62 %. The visceral vascular resistance increased significantly by 159 % in R_{CoA} and by 325 % in R_{MeA}. f_H and R_s were not significantly different from the untreated hypoxia values at the end of the hypoxic period (Fig. 3).

After bretylium treatment (*bretylium hypoxia*), the hypoxia-induced increases in P_{VA} and P_{DA} seen before treatment were abolished. Instead, P_{DA} was significantly decreased at the end of hypoxia after bretylium treatment. Cardiac output increased and the gastrointestinal blood flow decreased significantly (both q_{MeA} and q_{CoA} by 41 %) after bretylium treatment. However, there were no increases in R_{MeA} and R_{CoA} in the bretylium-treated fish. Hypoxia induced a significant reduction in the total systemic vascular resistance (by 47 %) in these animals (Fig. 3).

Following phentolamine treatment, both P_{VA} and P_{DA} were significantly decreased at the end of the hypoxic period (*phentolamine hypoxia*). These reductions were due to a decrease in the systemic vascular resistance. Both $\dot{Q}$ and V_S remained more or less unchanged throughout the period of hypoxia. q_{MeA} decreased significantly during hypoxia (28 %), and R_{MeA} as well as R_{CoA} decreased significantly during the same period (by 48 % and 45 %, respectively) (Fig. 3). Both adrenaline and noradrenaline levels increased significantly in the plasma during hypoxia (see Fig. 5).

Feeding

There was a statistically significant postfeeding increase in gut blood flow: q_{CoA} increased by 72 % and q_{MeA} by 42 %, while R_{CoA} and R_S decreased significantly by 31 % and 93 %, respectively (Fig. 4). Four hours after the injection of the α-adrenoceptor antagonist phentolamine (*phentolamine postfeeding*), a final recording of the variables was performed and statistically significant decreases, compared to the postfeeding recording, were found in P_{VA} (13 %), R_{MeA} (by 14 %) and R_S (by 0.4 %) (Fig. 4). No significant changes in the mesenteric or coelia

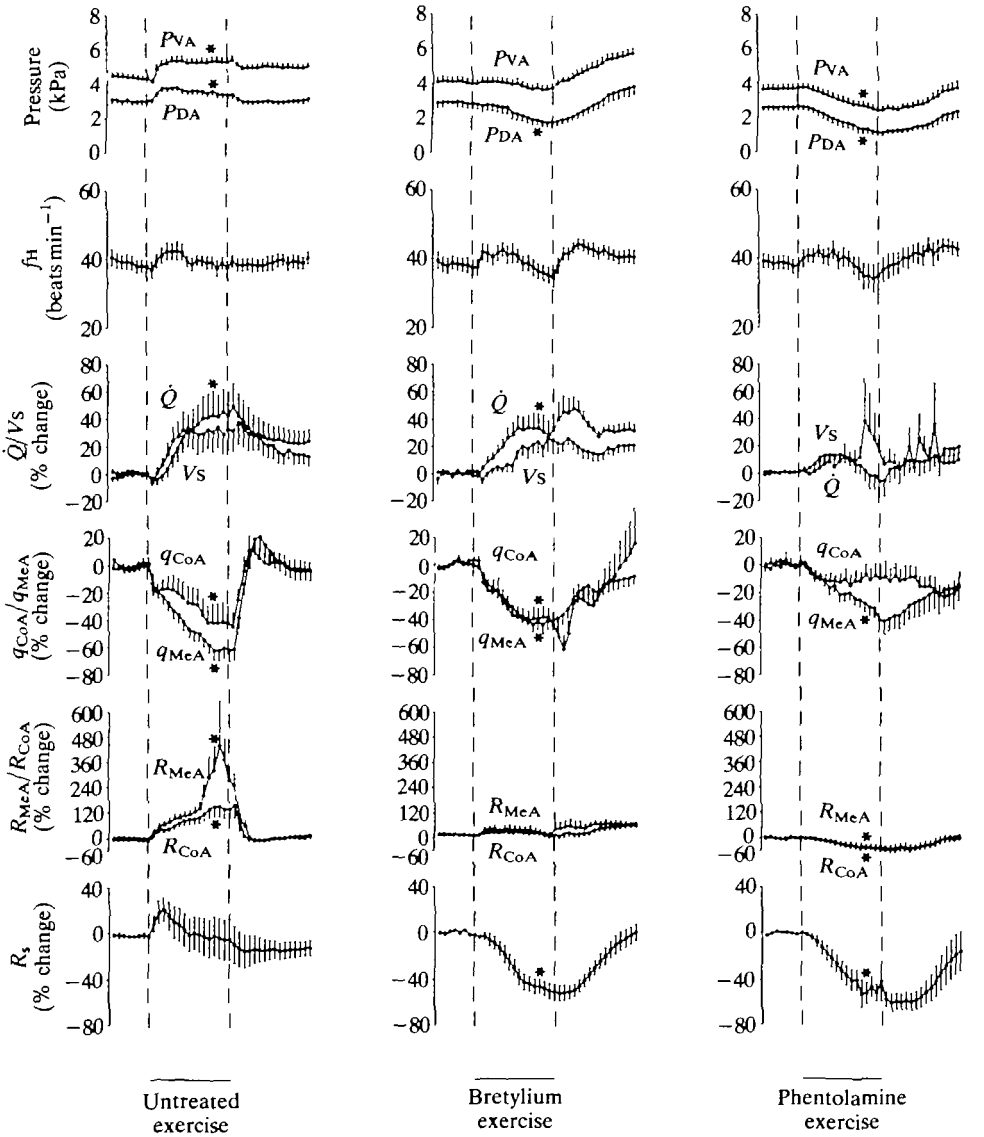

Fig. 3. A summary of cardiovascular responses to 8 min of exposure to hypoxia in the Atlantic cod in untreated animals (untreated hypoxia) in the same animals after treatment with bretylium (bretylium hypoxia) and after additional phentolamine treatment (phentolamine hypoxia), N=8–12. Mean values±S.E.M. are presented. Asterisks indicate statistically significant ($P \leq 0.05$) differences compared with normoxic values. Abbreviations are explained in the text.

artery blood flow could be observed after the injection of the α-adrenoceptor antagonist.

Plasma concentrations of catecholamines in the fish are presented in Fig. 5.

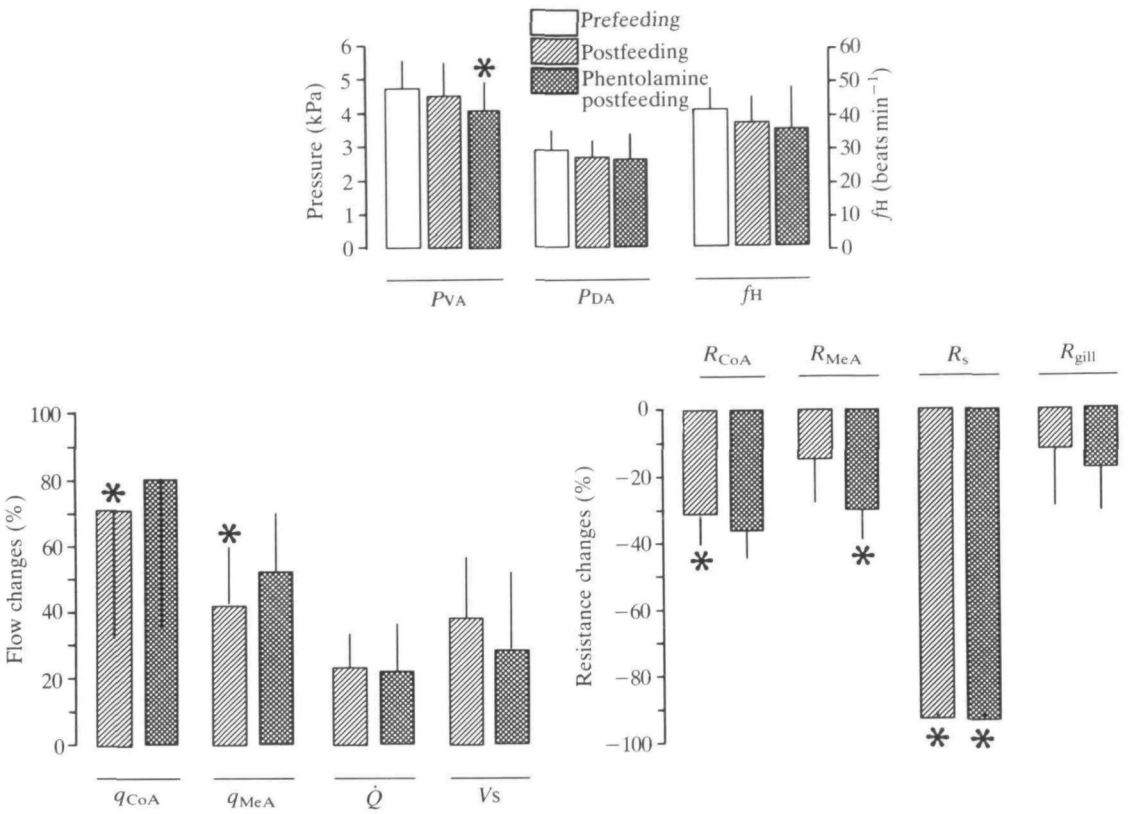

Fig. 4. Effects of feeding on circulatory variables in the cod. Bars show mean values+S.E.M., N=8–13. The flow and resistance values are shown as percentage changes from the untreated value (prefeeding). Asterisks indicate statistically significant ($P \leq 0.05$) differences between untreated (prefeeding) and 24-h postfeeding (postfeeding) and 24-h postfeeding compared to 4 h after phentolamine (phentolamine. postfeeding). Abbreviations are explained in the text.

Discussion

The present investigation is the first to present simultaneous measurements of the coeliac and mesenteric artery blood flow (q_{CoA}, q_{MeA}) in a teleost fish in association with exercise, hypoxia and feeding. In this study only relative changes in the ventral aortic blood flow were recorded, and for the discussion a $\dot{Q}$ value of 18–19 ml min^{-1} kg^{-1} will be used ($\dot{Q}$=19.2±0.9 ml min^{-1} kg^{-1}, Axelsson and Nilsson, 1986; 17.3±1.0 ml min^{-1} kg^{-1}, Axelsson, 1986, 19.2±2.3 ml min^{-1} kg^{-1}, Fritsche and Nilsson, 1989).

In the Atlantic cod, the coeliac and mesenteric arteries are of approximately the same diameter, and estimation of the volume flow in 10 animals by careful *in situ* calibration of the flow probes revealed similar blood flows in the two vessels (4.1 and 3.5 ml min^{-1} kg^{-1}, respectively). This represents about 40 % of cardiac output in the resting cod, assuming that $\dot{Q}$=18–19 ml min^{-1} kg^{-1}. In a study of the sea

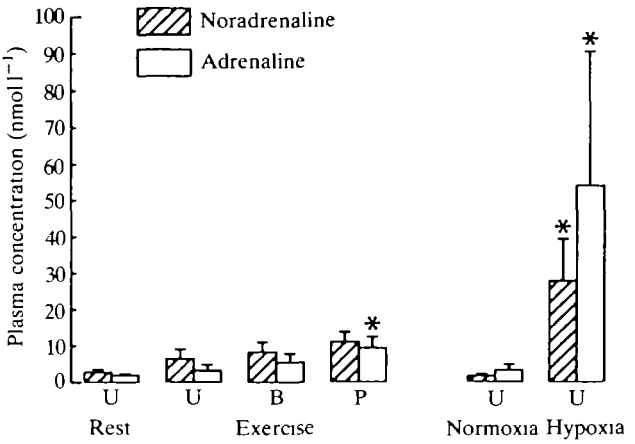

Fig. 5. Plasma concentrations of noradrenaline and adrenaline in resting and exercising fish, $N=9$, and in fish during normoxia, $N=8$. U, untreated fish; B, fish treated with bretylium; P, fish treated with phentolamine. Values are mean+S.E.M. Asterisks indicate values statistically different ($P \leq 0.05$) from rest values (exercise) or normoxia values (hypoxia).

raven (*Hemitripterus americanus*), a prefeeding blood flow of 2.9 ml min^{-1} kg^{-1} was observed in the coeliac artery, but no measurement of the mesenteric artery blood flow was performed (Axelsson *et al.* 1989). However, assuming that total gut blood flow is double that in the coeliac artery (the coeliac and mesenteric arteries are also of similar diameter in the sea raven), gut blood flow in the sea raven represents approximately 30 % of the cardiac output. These values are not dramatically different from the 27–30 % of cardiac output that is directed to the gut circulation *via* the coeliac and mesenteric arteries in resting, unfed mammals (Greenway, 1982).

Exercise

In the resting fish, injected bretylium produced a 32 % increase in the mesenteric artery blood flow, while no significant change in the coeliac artery blood flow could be detected. The subsequent phentolamine injection produced a further increase in the mesenteric artery blood flow (19 %), but again no change in the coeliac artery blood flow could be detected. These observations are compatible with the presence of an adrenergic tonus on the mesenteric vasculature in the cod, with both nervous and humoral components. Since the coeliac vasculature was unaffected by the adrenergic antagonists, non-adrenergic control of this vascular circuit is postulated. This contrasts with the conclusions from a study of the sea raven, where phentolamine produced a clear increase in coeliac artery blood flow, demonstrating adrenergic control of the coeliac artery vascular bed (Axelsson *et al.* 1989).

During exercise there was a reduction of the basal flow in the coeliac and

mesenteric arteries, caused by a marked increase in gut vascular resistance. This resistance increase was most pronounced at the onset of the exercise period, and a return towards the pre-exercise level was seen with time (Fig. 2). This may represent an autoregulatory 'escape reaction', a well-documented phenomenon in mammals, where it is most pronounced in the intestinal circulation but absent in skeletal muscles or adipose tissue (Greenway, 1984). The exact mechanisms behind this escape reaction are not known, but in mammals it is unaffected by the β-adrenoceptor antagonists atropine, antihistamines, indomethacin and naloxone (Greenway *et al.* 1976; Greenway, 1984).

In the cod, bretylium treatment abolished the observed 'escape reaction'; instead, the resistance increase in the coeliac and mesenteric artery persisted during the whole exercise period. This pattern was not altered by the subsequent α-adrenoceptor blockade with phentolamine. If the initial response is an escape reaction of the mammalian type, this finding suggests that an adrenergic nervous mechanism is involved in the development of this phenomenon in the cod.

The visceral vascular resistance also increased during exercise after bretylium treatment, causing a reduction in coeliac and mesenteric artery blood flow. After additional phentolamine treatment, only coeliac vascular resistance increased significantly, but the blood flow decreased in both vessels. The control of gut blood flow during exercise must therefore involve a non-adrenergic mechanism, in addition to an adrenergic one.

Exercise induced a decrease in the total systemic vascular resistance in the untreated fish, which may be explained by hyperaemia in the working muscles due to released metabolites. This phenomenon, observed and described by Gaskell (1880), has since been extensively studied in mammals (Whipp and Ward, 1982). An exercise-induced decrease in systemic vascular resistance has also been demonstrated in the rainbow trout (Kiceniuk and Jones, 1977; Randall and Daxboeck, 1982).

In the cod, bretylium treatment markedly potentiated the exercise-induced reduction in the systemic vascular resistance. This could be explained by an increase in the adrenergic nervous tone in the vasculature during exercise, which normally counteracts the effects of the local metabolite-induced hyperaemia. After bretylium treatment, this vasomotor activity ceases, revealing the full effect of the hyperaemia.

Hypoxia

During normoxia, injected bretylium and phentolamine did not change the visceral vascular resistance in the cod. These findings suggest the lack of adrenergic tonus in the gut vasculature in the undisturbed cod.

Although total cardiac output and dorsal aortic blood pressure increased during hypoxia, blood flow in the coeliac and mesenteric arteries decreased during the same period. This reduction in blood flow to the gut is due to increased visceral vascular resistance. After bretylium treatment, and also after additional phentolamine treatment, the systemic vascular resistance decreased during hypoxia

resulting in a fall in the ventral and dorsal aortic blood pressures. Bretylium also abolished the increase in visceral vascular resistance seen during hypoxia in the untreated fish, suggesting that an adrenergic nervous mechanism controls the mesenteric and coeliac vasculature in the cod during hypoxia. However, the gastrointestinal blood flow still decreased during hypoxia, owing to a fall in the dorsal aortic blood pressure.

The actual decrease in visceral vascular resistance during hypoxia after phentolamine treatment suggests adrenergic control of the gastrointestinal vasculature during hypoxia, with both nervous and humoral components.

This contrasts with the control of the gastrointestinal vasculature during exercise, when a clear adrenergic influence could be demonstrated only in the mesenteric vasculature.

Feeding

Feeding induced a flow increase of 72% in the coeliac artery, caused by a decrease in the coeliac vascular resistance, and of 42% in the mesenteric artery, which amounts to a postprandial gut blood flow of approximately 12 ml min^{-1} kg^{-1}. At the same time, $\dot{Q}$ increased by 23% and, assuming a control value of $\dot{Q}$ of 18 ml min^{-1} kg^{-1}, this represents an increase of 4.2 ml min^{-1} kg^{-1}. This increase is very similar to the increase in visceral blood flow (4.4 ml min^{-1} kg^{-1}). Postprandially the proportion of $\dot{Q}$ directed to the viscera increased from 40% to 52%. In mammals, the postfeeding increase in gut blood flow is in the range 30–150% (for references, see Fara, 1984). The hyperaemia is accomplished either by an increase in cardiac output or by a redistribution of blood away from other vascular beds to the gut (Fara, 1984). In mammals, the hyperaemia is localized to those parts of the gut containing food, and an increase in coeliac artery blood flow precedes the flow increase in the mesenteric arteries (Biber, 1974; Gallavan *et al.* 1980). Further work is needed in fish to establish whether the more pronounced flow increase in the coeliac artery (72%) compared to that in the mesenteric artery (42%) is due to a similar sequential hyperaemia.

The gastric emptying time is dependent on temperature, food quality, surface area of the food, amount of food ingested (and thus the degree of distension of the stomach) and the secretory surface of the stomach. In mammals, blood flow to the stomach and intestine starts to increase within 3–5 min after the arrival of food, and the hyperaemia lasts for 4–6 h, depending on the type of food ingested (Fara, 1984). In fish and other ectothermic animals, the digestion rate is low and gastric emptying time is long compared with that in endothermic mammals. In the cod, gastric emptying times of around 12–45 h have been reported at 6–10°C (Tyler, 1970; Jones, 1974), but the amount of food used in these studies was small (0.2–2.7% kg^{-1} body mass). In the present study, only partial digestion of the food was seen 29 h after feeding (*post mortem*).

No change in blood flow to the gut was seen in the fed fish after the injection of the α-adrenoceptor antagonist phentolamine, but there was a decrease in the

mesenteric vascular resistance. This contrasts with the results from the sea raven study, where phentolamine caused an increase in the coeliac artery flow from 5.8 ml min^{-1} kg^{-1} (postprandial) to 11.3 ml min^{-1} kg^{-1} (Axelsson *et al.* 1989).

In the cod, it has been shown that both substance P and vasoactive intestinal polypeptide (VIP) increase the gastrointestinal blood flow and that there exists a cholinergically mediated vasoconstrictor mechanism in the coeliac artery vascular circuit (Jensen *et al.* 1990). In mammals, it has been suggested that cholecystokinin (CCK) may be the active mediator of the mesenteric postprandial hyperaemia (Fara, 1984), but many other gastrointestinal hormones cause vasodilatation in the small intestine of mammals, for instance secretin, gastrin, glucagon, VIP, opiate agonists (morphine, endorphins, met-enkephalin), neurotensin, substance P and gastric inhibitory polypeptide (Chou *et al.* 1984). The large number of putative neurotransmitters and gut hormones with a potential role in gut vascular control opens up a wealth of possible mechanisms, and further studies are obviously needed to elucidate the mechanisms involved in the regulation of the gut blood supply during exercise, hypoxia and feeding in fish.

It is clear from this study that the control of the gastrointestinal vasculature varies with the stimulus. During hypoxia, adrenergic control of the gastrointestinal vasculature with both nervous and humoral components was found, while the control of gut blood flow during exercise and feeding also involves an additional non-adrenergic mechanism.

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References

AXELSSON, M. (1988). The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod, *Gadus morhua* at rest and during non-exhaustive exercise. *J. exp. Biol.* **137**, 287–303.

AXELSSON, M., DRIEDZIC, W. R., FARRELL, A. P. AND NILSSON, S. (1989). Regulation of cardiac output and gut blood flow in the sea raven, *Hemitripterus americanus*. *Fish Physiol. Biochem.* **6**, 315–326.

AXELSSON, M. AND NILSSON, S. (1986). Blood pressure regulation during exercise in the Atlantic cod, *Gadus morhua*. *J. exp. Biol.* **126**, 225–236.

BIBER, B. (1974). Vasodilator mechanisms in the small intestine. *Acta physiol. Scand.* **90** (Suppl. 401), 1–31.

CHOU, C. C., MANGINO, M. J. AND SAWMILLER, D. R. (1984). Gastrointestinal hormones and intestinal blood flow. In *Physiology of the Intestinal Circulation* (ed. A. P. Shepherd and D. N. Granger), pp. 121–130. New York: Raven Press.

DAXBOECK, C. (1981). A study of the cardiovascular system of fish (*Salmo gairdneri*) at rest and during swimming exercise. PhD thesis, University of British Columbia, Vancouver.

DAXBOECK, C. AND HOLETON, G. F. (1978). Oxygen receptors in the rainbow trout, *Salmo gairdneri*. *Can. J. Zool.* **56**, 1254–1259.

DONALD, E. D. (1980). Role of autonomic nerves in the cardiovascular response to exercise in the dog. In *Exercise Bioenergetics and Gas Exchange* (ed. P. Cerretelli and B. J. Whipp), pp. 267–274. Amsterdam: Elsevier, North-Holland Biomedical Press.

FARA, J. W. (1984). Postprandial mesenteric hyperemia. In *Physiology of the Intestinal Circulation* (ed. A. P. Shepherd and D. N. Granger), pp. 99–106. New York: Raven Press.

FARRELL, A. P. (1984). A review of cardiac performance in the teleost heart: intrinsic and humoral regulation. *Can. J. Zool.* **62**, 523–536.

FRITSCHE, R. AND NILSSON, S. (1989). Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. *Exp. Biol.* **48**, 153–160.

FRITSCHE, R. AND NILSSON, S. (1990). Autonomic nervous control of blood pressure and heart rate during hypoxia in the cod, *Gadus morhua*. *J. comp. Physiol.* B **160**, 287–292.

GALLAVAN, R. H., CHOU, C. C., KVIETYS, P. R. AND SIT, S. P. (1980). Regional blood flow during digestion in the conscious dog. *Am. J. Physiol.* **238**, H220–H225.

GASKELL, W. H. (1880). On the tonicity of heart and blood vessels. *J. Physiol., Lond.* **4**, 48–75.

GEHRKE, P. C. AND FIELDER, D. R. (1988). Effects of temperature and dissolved oxygen on heart rate, ventilation rate and oxygen consumption of spangled perch, *Leioptherapon unicolor*. *J. comp. Physiol.* **157**, 771–782.

GREENWAY, C. V. (1982). Mechanisms and quantitative assessment of drug effects on cardiac output with a new model of the circulation. *Pharmac. Rev.* **33**, 213–251.

GREENWAY, C. V. (1984). Neural control and autoregulatory escape. In *Physiology of the Intestinal Circulation* (ed. A. P. Shepherd and D. N. Granger), pp. 61–71. New York: Raven press.

GREENWAY, C. V., SCOTT, G. D. AND ZINK, J. (1976). Sites of autoregulatory escape of blood flow in the mesenteric vascular bed. *J. Physiol., Lond.* **259**, 1–12.

HALLMAN, H., FARNEBO, L.-O., HAMBERGER, B. AND JONSON, G. (1978). A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection. *Life Sci.* **23**, 1049–1052.

HOLETON, G. F. AND RANDALL, D. J. (1967). Changes in blood pressure in the rainbow trout during hypoxia. *J. exp. Biol.* **46**, 297–305.

HOLM, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* **6**, 65–70.

JENSEN, J., AXELSSON, M. AND HOLMGREN, S. (1990). Effects of substance P and vasoactive intestinal polypeptide on gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. *J. exp. Biol.* **156**, 361–373.

JONES, R. (1974). The rate of elimination of food from the stomach of haddock, *Melanogrammus aeglefinus*, cod, *Gadus morhua* and whiting, *Merlangius merlangus*. *J. Cons. int. Explor. Mer.* **35**, 225–298.

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

LAURENT, P., HOLMGREN, S. AND NILSSON, S. (1983). Nervous and humoral control of the fish heart: structure and function. *Comp. Biochem. Physiol.* **76**A, 524–542.

NILSSON, S. (1983). *Autonomic Nerve Function in the Vertebrates*. Berlin, Heidelberg, New York: Springer-Verlag.

NILSSON, S. AND HOLMGREN, S. (1989). Novel neurotransmitter in the autonomic nervous system of nonmammalian vertebrates. *Pharmac. Ther.* **41**, 257–287.

ORWELL, L. B., BLACKMON, J. R. AND BRUCE, R. A. (1964). Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man. *J. clin. Invest.* **43**, 1677–1690.

RANDALL, D. J. AND DAXBOECK, C. (1982). Cardiovascular changes in the rainbow trout (*Salmo gairdneri* Richardson) during exercise. *Can. J. Zool.* **60**, 1135–1140.

RUSHMER, R. F., FRANKLIN, D. L., VAN CITTERS, R. L. AND SMITH, O. A. (1961). Changes in peripheral blood flow distribution in healthy dogs. *Circulation Res.* **9**, 675–687.

SMITH, D. G. (1978). Neural regulation of blood pressure in rainbow trout (*Salmo gairdneri*) *Can. J. Zool.* **56**, 1678–1683.

SMITH, D. G., WAHLQVIST, I., NILSSON, S. AND ERIKSSON, B.-M. (1985). Nervous control of the blood pressure in the Atlantic cod, *Gadus morhua*. *J. exp. Biol.* **117**, 335–347.

TYLER, A. V. (1970). Rates of gastric emptying in young cod. *J. Fish. Res. Bd Can.* **27**, 1177–1189.

VATNER, S. F., FRANKLIN, D., HIGGINS, C. B., PATRICK, T., WHITE, S. AND VAN CITTERS, R. L. (1971). Coronary dynamics in unrestrained conscious baboons. *Am. J. Physiol.* **221**, 1396–1401.

WAHLQVIST, I. AND CAMPBELL, G. (1988). Autonomic influences on heart rate and blood pressure in the toad, *Bufo marinus*, at rest and during exercise. *J. exp. Biol.* **134**, 377–396.

WHIPP, B. J. AND WARD, S. A. (1982). Cardiopulmonary coupling during exercise. *J. exp. Biol.* **100**, 175–193.