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Sympathetic, parasympathetic and enteric regulation of the gastrointestinal vasculature in rainbow trout (*Oncorhynchus mykiss*) under normal and postprandial conditions

Henrik Seth* and Michael Axelsson

Department of Zoology, University of Gothenburg, Box 463, S-405 30 Gothenburg, Sweden

*Author for correspondence (henrik.seth@zool.gu.se)

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SUMMARY

The control of the gastrointestinal hyperemia that occurs after feeding in most animals is of fundamental importance for the subsequent absorption, metabolism and redistribution of nutrients. Yet, in fish, it has received little attention and the nature of it is far from clear. We sought to investigate the importance of extrinsic and intrinsic innervation of the gastrointestinal tract in the regulation of gastrointestinal blood flow in rainbow trout (*Oncorhynchus mykiss*). The contribution of the extrinsic innervation, i.e. by the sympathetic and the parasympathetic nervous system, was examined by comparing the response to the injection of a predigested nutrient diet into the proximal intestine of untreated fish with the response in fish in which the splanchnic and vagal innervation of the gut had been removed. We also injected the predigested nutrient diet into anaesthetized fish treated with tetrodotoxin that would block the intrinsic innervation of the gut (i.e. enteric nervous system). Our results confirm the notion that the sympathetic portion of the extrinsic innervation maintains the basal vascular tone, but neither the splanchnic nor the vagal innervation is fundamental to the postprandial hyperemia. However, the tetrodotoxin treatment completely abolished the postprandial hyperemia, indicating the importance of the enteric nervous system. In conclusion, it seems as though the enteric nervous system is essential to the regulation of the postprandial hyperemia, and that the extrinsic innervation is involved mainly in the regulation of gastrointestinal blood flow under normal conditions and in response to central coordination with other organs.

Key words: vagus nerve, splanchnic nerve, teleost, extrinsic innervation, intrinsic innervation, enteric.

INTRODUCTION

The control of gastrointestinal blood flow, especially after feeding, has received much attention in mammals, but details still remains elusive despite the fact that several studies have focused directly on the cardiovascular response to feeding; this has also been extensively reviewed (Chou and Coatney, 1994; Chou et al., 1984; Granger and Kvietys, 1981; Granger et al., 1980; Kvietys and Granger, 1982; Matheson et al., 2000). So far it has been shown in mammalian species that mechanical stimuli might contribute (Biber, 1973), but chemical stimuli are almost certainly more important in the control of the gastrointestinal blood flow control. The chemical stimulus is dependent on the composition of the feed and it has been noted that different nutrients are not equally essential in inducing the postprandial hyperemia (Chou et al., 1972; Chou et al., 1978; Kvietys et al., 1981; Siregar and Chou, 1982). The exact mechanism controlling the hyperemia in mammals is under debate but may involve direct effects from the absorbed nutrients on the vasculature (Chou et al., 1985), endocrine factors (Biber, 1973; Chou et al., 1977; Chou et al., 1984; Fara et al., 1972), non-metabolic vasoactive factors (Chou et al., 1989; Chou and Siregar, 1982; Sawmiller and Chou, 1988; Sawmiller and Chou, 1990), metabolic vasoactive factors (Bohlen, 1980a; Bohlen, 1980b; Bohlen, 1998a; Pawlik et al., 1980) and neural mechanisms (Biber, 1973; Kato et al., 1989; Takagi et al., 1988). There are, however, large variations in mammals reported in the literature, which, to some extent, can be explained by large interspecies differences. Nevertheless, the results indicate that metabolic factors pertaining to a change in the partial pressure of oxygen or the osmolarity of the gut are of major importance in triggering the postprandial gastrointestinal hyperemia (Bohlen, 1980a; Bohlen, 1980b; Bohlen, 1982; Bohlen, 1998a). However, this does not exclude a possible importance of neural mechanisms in, for example, controlling the response to a change in the partial pressure of oxygen within the gastrointestinal tract (Surprenant, 1994; Vanner and Surprenant, 1996).

In fish, little is known about most aspects of the postprandial regulation of gastrointestinal blood flow and little attention has been directed to a possible neural regulation of gastrointestinal blood flow in teleosts. We have previously shown, in two separate teleost species, that despite the obvious differences between mammalian and piscine species, there are numerous similarities in the postprandial cardiovascular response. For instance, the mechanical distension that occurs as food enters the stomach induces a pressor response (i.e. increased dorsal aortic blood pressure) that may facilitate an efficient shunting of blood from the systemic circulation to the gastrointestinal tract when hydrolyzed food components induce a subsequent intestinal hyperemia (Seth and Axelsson, 2009; Seth et al., 2008). Furthermore, we have also shown that the nutrient components are not equally important and that a balanced diet, resembling the natural diet, induces the most profound hyperemia (Seth et al., 2009). The reason for this and the mechanisms of control behind the hyperemia remain to be established in fish.

It is probable that the postprandial gastrointestinal hyperemia is influenced by, or depends on, neural components, either extrinsic (sympathetic and/or parasympathetic) or intrinsic (enteric) to the gastrointestinal tract. A few studies have revealed an extrinsic neural component that directly influences the postprandial hyperemia in rats (Rozsa and Jacobson, 1989) or has indirect effects *via* cholecystokinin in cats (Biber et al., 1974),

as well as *via* a non-cholinergic, non-adrenergic neural mechanism in mongrel dogs (Kato et al., 1989; Takagi et al., 1988). Other studies in mammals have, however, concluded that there is no neural component or it is at least of minor importance compared with the metabolic component (Nyhof and Chou, 1981; Nyhof and Chou, 1983; Nyhof et al., 1985; Vanner and Surprenant, 1996). Even less is known about a possible role for the enteric nervous system (Furness, 2006; Olsson and Holmgren, 2009; Olsson et al., 2009), intrinsic to the gut, in the modulation of the hyperemic response although it has been implicated as possible factor in connecting the metabolic response to the status of the vasculature (Bohlen, 1998a; Bohlen, 1998b).

The aim of this study was to examine the involvement of the extrinsic and intrinsic innervation of the gastrointestinal tract, i.e. the sympathetic, parasympathetic and enteric innervation, respectively, in the control of the postprandial hyperemia in rainbow trout. This was done by sectioning the vagal and splanchnic innervation of the gastrointestinal tract, as well as by blocking the enteric portion with the voltage-gated sodium-channel-inhibitor tetrodotoxin (TTX).

MATERIALS AND METHODS

Experimental animals

Rainbow trout (*Oncorhynchus mykiss* Walbaum) ranging in size from 400 to 635 g (478±7 g; *N*=65) were acquired from a local hatchery (Antens laxodling, AB, Gothenburg, Sweden). The fish were held in 1–2 m^3 fiberglass tanks supplied with aerated freshwater (10–11°C) from the re-circulating departmental water system and fed dry trout pellets at regular intervals. The photoperiod was adjusted to 12 h:12 h light:dark. When fish arrived, they were left to acclimatize to the new environment for at least 2 weeks prior to any experimental procedures. Ethical permit 13/2007 from the animal ethics committee of Gothenburg covered all experiments reported here.

Characterization of the neural input to the gastrointestinal tract

The extrinsic innervation of the gastrointestinal tract of the rainbow trout was examined in anaesthetized fish (*N*=8) under a dissecting microscope (Fig. 1) and this confirmed previous results obtained in brown trout (*Salmo trutta*) (Burnstock, 1959) using ocular inspection of the gastrointestinal tissues stained with osmium tetroxide (OsO_4) *ex vivo*.

Surgical procedures

In vivo measurements of cardiovascular variables in untreated and denervated fish

Fish were fasted for a week prior to surgery. Individual fish were anaesthetized in water containing MS-222 (tricaine; 150 mg l^{-1}) buffered with sodium bicarbonate (300 mg l^{-1}) and placed on a surgery table covered with wet rubber foam. The gills were continuously irrigated with well aerated fresh water (10°C) containing MS-222 (75 mg l^{-1}) buffered with sodium bicarbonate (150 mg l^{-1}).

In order to inject nutrients directly into the proximal part of the intestine, we used a previously described method (Seth et al., 2009). In short, a small dorsoventral incision was made in the body wall 2 cm anterior to the pelvic fin and a plastic tube was introduced into the stomach via the mouth in order to access the stomach by moving it towards the incision. A small double lumen Fogarty embolectomy catheter (12TLW805F35; V-Tech AB, Göteborg, Sweden) was introduced into the proximal part of the intestine, through the stomach, and secured in the intestine by the inflatable bubble at the end of the catheter. The stomach wall was closed around the catheter with a purse-string suture (4/0 silk). Finally the body wall was closed using uninterrupted running sutures (3/0 silk) and the catheter was secured at the back of the fish with two sutures.

Relative changes in cardiac output ($\dot{Q}$) and gastrointestinal blood flow ($\dot{Q}_{cma}$) were measured using Doppler flow probes placed around the ventral aorta (i.d.: 1.8 mm) and the coeliacomesenteric artery (i.d.:1.0–1.2 mm) just proximal to the bifurcation into the gastric and intestinal artery (Seth et al., 2008). Both flow probes were custom-made from Perspex and equipped with 20 MHz Doppler crystals (0.5 mm; Iowa Doppler products, Iowa City, IA, USA).

The gastrointestinal tract was denervated by cutting the vagi bilaterally. The vagus nerve was accessed through a small (0.5–1 cm) dorsoventral incision in the tissue connecting the fourth gill arch and the cleithrum. Just below the connective tissue the vagi were immediately visible, running parallel to the duct of Cuvier. The branches of the vagus innervating the upper gastrointestinal tract (Fig. 1) were identified as they curve caudally and disappear under the duct of Cuvier. The gastrointestinal branch of vagus was bilaterally sectioned making sure that no part of this branch still remained intact. The incision was then closed with silk sutures (4/0).

The splanchnic nerve was accessed when placing the flow probe on the coeliacomesenteric artery. As shown in Fig. 1, the nerve runs

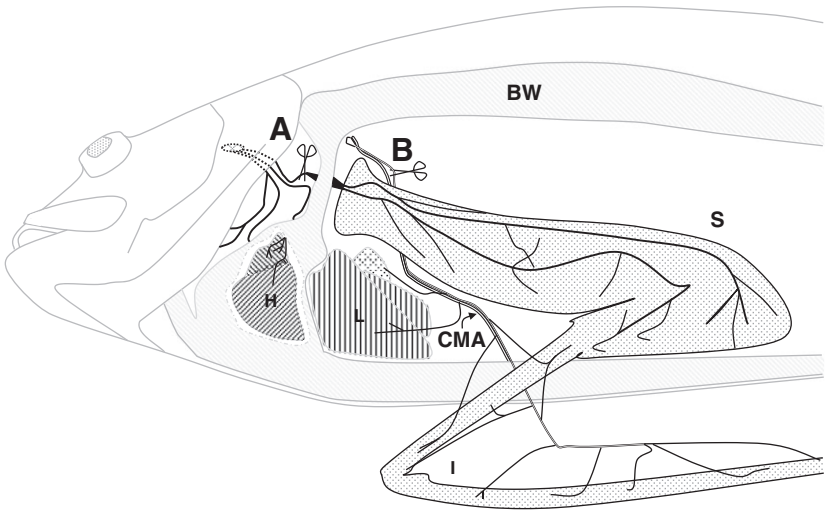

Fig. 1. Left lateral view of a rainbow trout (*Oncorhynchus mykiss*), showing the innervation of the gastrointestinal tract *via* the vagus nerve (A) and the splanchnic nerve (B). Scissors indicate the point of sectioning. BW, body wall; CMA, coeliacomesenteric artery; H, heart; I, intestine; L, liver; S, stomach.

parallel to the coeliacomesenteric artery. The splanchnic nerve was sectioned just before the bifurcation of the coeliacomesenteric artery, making sure that no part still remained intact.

In vivo measurement of blood flow through the coeliacomesenteric artery

To evaluate the basal flow through the coeliacomesenteric artery before and after sectioning of the splanchnic nerve, fish were instrumented with a transit-time flow probe (0.7 mm V-probe; Transonic Systems Inc., Ithaca, NY 14850, USA). The probe was placed around the artery in much the same way as was described above for the Doppler flow probe. To measure dorsal aortic pressure and thus be able to calculate the basal resistance of the coeliacomesenteric vascular circuit, the dorsal aorta was cannulated *via* the roof of the mouth as previously described (Axelsson and Fritsche, 1994). The sectioning of the splanchnic nerve was performed as detailed above.

In situ measurement of cardiovascular variables in untreated and TTX-treated fish

To assess the effect of TTX treatment on several cardiovascular variables we anaesthetized fish and instrumented the animals with Doppler flow probes on the ventral aorta and the coeliacomesenteric artery as describe above. To measure dorsal aortic pressure and inject TTX, the dorsal aorta was cannulated *via* the roof of the mouth as previously described (Axelsson and Fritsche, 1994). A pre-digested diet was injected into the proximal intestine *via* a cannula as describe above. Fish were kept under anesthesia on the operating table during the entire experimental protocol.

Experimental diet

A balanced diet (50% protein, 25% fat, 15% carbohydrates and ash 8%) was prepared as previously described (Seth et al., 2009) using fish protein (90% pure; Marine Bioproducts, Storebro, Norway), fish oil (99% pure; Marine Bioproducts) and glucose [D-(+)-glucose–G7528; Sigma-Aldrich, Stockholm, Sweden), and stored overnight at 3–4°C (Seth et al., 2009). The water content was 70–80%, and pH and osmolarity were adjusted to between 7.3–7.4 and 380–400 mOsm, respectively, to mimic the normal conditions of the rainbow trout proximal intestine (Bucking and Wood, 2006). The balanced diet was predigested with physiological concentrations of bile salts obtained from the respective fish during surgery (0.07 ml g^{-1} diet) using a fine cannula [27 gauge × 3/4″ (2 cm)] and pancreatic enzymes (Pancreatin–P8096, Sigma-Aldrich) containing lipases and proteases such as trypsin in sufficient amounts.

Experimental protocols

In vivo measurements of cardiovascular variables in untreated and denervated fish

Untreated (N=8) and denervated fish (N=7) were allowed 48 h of recovery post-surgery before experimentation. They were held in opaque chambers supplied with aerated freshwater (10–11°C) from the departmental re-circulating water system. Basal cardiovascular variables were recorded for 1 h before the injection of the pre-digested nutrient solution (1.2 ml kg^{-1}). Every injection was followed by an equal amount of saline to flush the catheter dead-space. Cardiovascular variables were thereafter measured for ~12 h. Control experiments (N=4) were also conducted where saline (1.2 ml kg^{-1}) was injected instead of the pre-digested nutrient solution. After the experiments the position of the catheter and the integrity of the stomach and proximal intestine were confirmed. The bilateral vagotomy was also assessed by visual inspection to verify that no gastrointestinal branches of the vagus still remained intact.

In vivo measurements of basal cardiovascular variables with atropine treatment

Another group of untreated rainbow trout (N=4) were allowed 48 h of post-surgical recovery prior to experimentation. Basal cardiovascular variables were recorded for 1 h before the injection of atropine (1.2 mg kg^{-1}) into the proximal intestine. Cardiovascular variables were thereafter measured for ~6 h.

In vivo measurement of absolute blood flow through the coeliacomesenteric artery

Fish (N=8) equipped with transit-time flow probes were allowed to recover for at least 24 h before basal gastrointestinal blood flow were measure for several hours. They were then lightly anaesthetized and swiftly transferred to the operating table, where the splanchnic nerve was cut. After returning the fish to the opaque experimental chambers they were allowed to recover for another 24 h before basal gastrointestinal blood flow was measured again.

In another group of fish (N=8), four animals were denervated immediately while the other four animals were sham denervated. After a recovery period of 24 h, basal gastrointestinal blood flow was measured and a comparison was made between the two groups. This was done to test for any confounding effects of the second surgery in the paired experimental protocol.

In situ measurement of cardiovascular variables in untreated and TTX-treated fish

Fish were allowed to stabilize on the surgical table for at least 1–2 h after the surgery. During the entire experimental protocol the gills were continuously irrigated with aerated fresh water (10°C) containing MS-222 (75 mg l^{-1}) buffered with sodium bicarbonate (150 mg l^{-1}). In one group (N=8) the normal cardiovascular variables were recorded for 1 h before the injection of a balanced nutrient diet as described above. The studied variables were then recorded for another 3–4 h. In a second group, the control group (N=7), the protocol was repeated but saline was substituted for the pre-digested nutrient diet. In the third group (N=7), TTX (1 ml kg^{-1}, 4 μM) was injected after the initial recording of normal cardiovascular variables. The fish were then left to stabilize for another hour, by which time the cardiovascular variables had returned to pre-TTX-administered values. The nutrient diet was then injected and the variables were recorded for another 3–4 h.

The correct dosage was obtained using *in-situ* perfused hearts (N=4) as previously described by Seth et al. (Seth et al., 2010) and the concentration of TTX in the perfusate was increased in a stepwise manor until a change in the contractility of the heart was noticed. Additional *in vivo* trials on anaesthetized fish (N=4) were also conducted. At the dosage used in the experimental protocol TTX had limited effects on the heart with no change in heart rate and cardiac output, while completely abolishing spontaneous bodily movements.

Drugs

Tetrodotoxin (TTX) was obtained from Sigma-Aldrich (T-8024 99% HPLC, 89552 Steinheim, Germany) and diluted in 0.9% saline. Atropine (atropine sulfate salt, ≥97%, A-0257) and adrenaline [(–)-epinephrine (+)-bitartrate salt, E4375) was obtained from Sigma-Aldrich (Sigma-Aldrich, 3050 Spruce St., St Louis, USA) and diluted in 0.9% saline.

Data acquisition and statistics

Relative changes in blood flow were recorded using a directional pulsed Doppler flow meter (model 545C-4, The University of Iowa, Iowa, USA). Signals from the Doppler flow meter were fed into a PowerLab system (PowerLab 8/30, ADInstruments Pty Ltd, Castle Hill, Australia) connected to a computer running Chart software (Chart 5.4.1. ADInstruments Pty Ltd). Heart rate (f_H) was obtained from the phasic cardiac output ($\dot{Q}$) signal and cardiac stroke volume (V_S) was calculated as $V_S=\dot{Q}\,h^{-1}$. Absolute blood flows were recorded using a transit-time flow meter (T206, Transonic Systems Inc., Ithaca, NY 14850, USA) and normalized to body mass. The vascular resistance of the splanchnic circulation was calculated as $R_{cma}=(P_{da}-P_{ven})/\dot{Q}_{cma}$, where R_{cma} is the resistance of the gastrointestinal vasculature, P_{da} is the dorsal aortic blood pressure and P_{ven} is the central venous blood pressure. Three assumptions were made in these calculations: (1) the driving force through the coeliacomesenteric artery is the dorsal aortic blood pressure; (2) the venous pressure is zero and does not change; and (3) the blood viscosity does not change during the experimental protocol. Blood flow and stroke volume data were converted to percentage values with the initial basal control value set to 100%. Data were collected at 20 Hz and all reported values are means ± s.e.m.

The baseline values before the injection of the nutrient solution were compared with the values after the injection by a repeated measures ANOVA followed by Dunnet's *post-hoc* test to analyze for significant differences from the control. To control for a difference between the untreated and the denervated group, individual time points were analyzed using a two-sample *t*-test, assuming equal variance. Multiple comparisons were corrected for using the Holm–Bonferroni algorithm. All statistical comparisons were performed using raw unformatted data. A significant difference from the baseline (*) and between the treatments (†) was assumed when $P<0.05$, and $P<0.01$ (**).

RESULTS

Extrinsic regulation of postprandial gut blood flow

After the injection of a predigested diet into the proximal stomach of untreated rainbow trout there was a significant increase both in gut blood flow ($\dot{Q}_{cma}$) and in cardiac output ($\dot{Q}$) compared with control as well as basal values. The increase in $\dot{Q}$ at 30 min was 34.5±7% (Fig. 2A), which would be more than enough to sustain the 17.2±6% increase in $\dot{Q}_{cma}$ (Fig. 2B). Gastrointestinal blood flow increased further and peaked at around 2 h post-injection, with an increase of 29.4±3%. The peak increase in $\dot{Q}$ (36±11%) at 2 h was mediated by a significant increase in stroke volume (V_S; 28.3±10%; Fig. 2D) as heart rate (f_H) did not change significantly during the procedure (Fig. 2C).

When injecting the predigested diet into the proximal stomach of rainbow trout, where the extrinsic innervation had been removed by sectioning the vagi and the splanchnic nerve, there was still a significant increase in gut blood flow. The response was, however, delayed compared with the untreated fish. After 1 h, $\dot{Q}_{cma}$ had increased by 20.5±4% (Fig. 2A). There was no significant increase in either $\dot{Q}$ (9.3±4%; Fig. 2B) or V_S (9±4%; Fig. 2C), at 1 h. In concurrence with the untreated fish, the maximal increase in $\dot{Q}_{cma}$ (25.3±10%) occurred at 2 h post-feeding, and was maintained *via* a significant increase in $\dot{Q}$ (24.5±9%) due to a 24.9±8% increase in V_S. No change in f_H was recorded, but when comparing the untreated fish with the denervated fish there was a small but significant increase in heart rate (Fig. 2D), possibly baroreflex

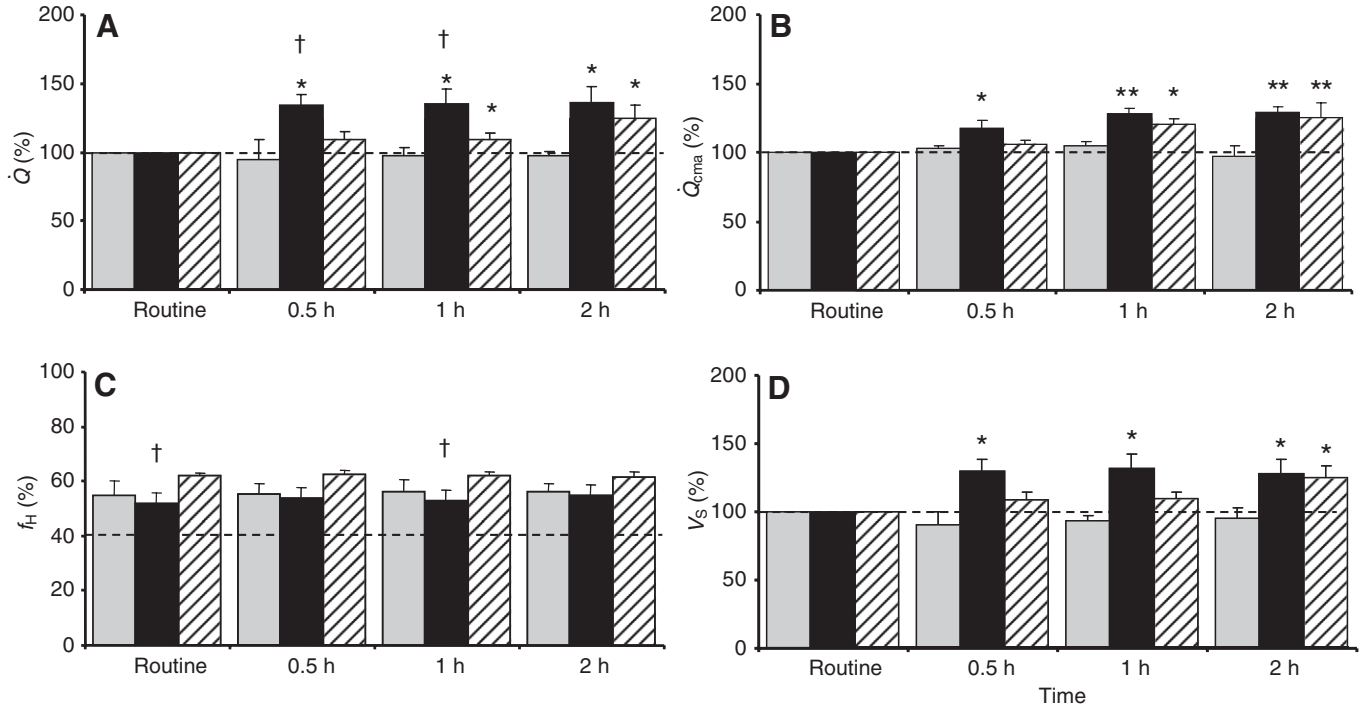

Fig. 2. *In vivo* physiological measurements in rainbow trout (*Oncorhynchus mykiss*). (A) Cardiac output ($\dot{Q}$), (B) gastrointestinal blood flow ($\dot{Q}_{cma}$) at the coeliacomesenteric artery, (C) heart rate (f_H) and (D) stroke volume (V_S). Values are means ± s.e.m. Control animals (grey bars) received an injection of saline (1.2 ml kg^{-1}) into the proximal intestine (N=5, 486±24 g). Untreated animals (N=8, 483±16 g) received an injection of a predigested diet (1.2 ml kg^{-1}) into the proximal intestine (black bars). Denervated animals, in which the two vagus nerves and the splanchnic nerve had been sectioned (N=8, 51±20 g), also received an injection of a predigested diet (1.2 ml kg^{-1}) into the proximal intestine (hatched bars). The dashed line indicates 100% for the normalized values which is arbitrarily set to 40 beats min^{-1} for f_H. Asterisks denote a significant difference from both the normal values and the control (*P<0.05 and **0.01, respectively); †a significant difference between the untreated and the denervated group (P<0.05).

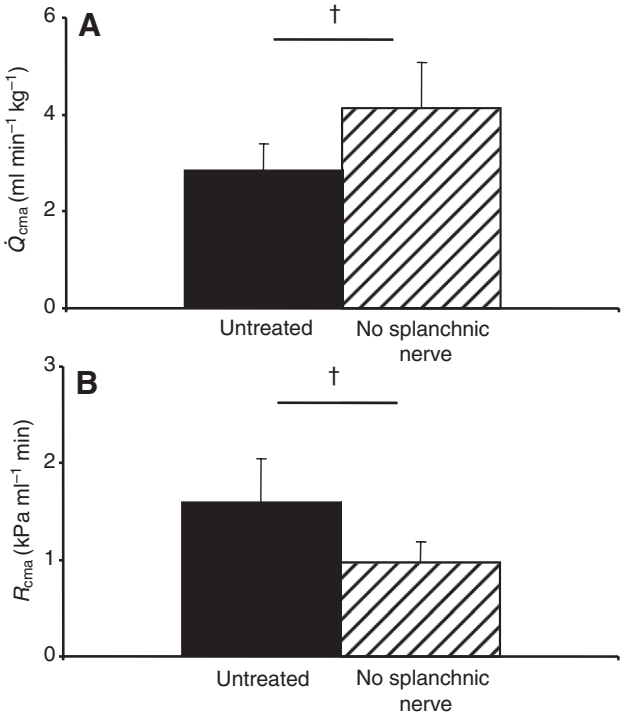

Fig. 3. *In vivo* measurements in rainbow trout (*Oncorhynchus mykiss*) of (A) gastrointestinal blood flow at the coeliacomesenteric artery ($\dot{Q}_{cma}$) and (B) vascular resistance of the coeliacomesenteric vascular system (R_{cma}). Values are means ± s.e.m. $\dot{Q}_{cma}$ and R_{cma} were recorded during normal conditions before (black bars) and after (hatched bars) the splanchnic nerve had been sectioned in animals weighing 491±27 g (N=8). †A significant difference between the untreated and the denervated group (P<0.05).

mediated because of a lowered blood pressure as a consequence of the lowered peripheral resistance. None of the measured cardiovascular variables changed after the injection of saline.

Extrinsic regulation of basal vascular tone of the gastrointestinal tract

The basal gastrointestinal blood flow (normalized to per kg) in untreated rainbow trout was 3.0±0.5 ml min^{-1} kg^{-1} (Fig. 3A). With a dorsal aortic pressure of 3.6±0.2 kPa, the calculated resistance of the gastrointestinal vasculature was 1.6±0.4 kPa ml^{-1} min^{-1} kg^{-1} (Fig. 3B). When the splanchnic nerve was sectioned there was a significant (P<0.05) increase in the gastrointestinal blood flow to 4.6±0.8 ml min^{-1} kg^{-1} (63±25%), due to a decrease in the resistance of the gastrointestinal vasculature to 1.0±0.2 kPa ml^{-1} min^{-1} kg^{-1} (–33±10%) as seen in Fig. 3A,B. The dorsal aortic pressure decreased to 3.4±0.2 kPa. A significant decrease in the vascular resistance was also seen in the unpaired group (data not shown).

The injection of atropine into the proximal intestine caused a profound increase in both cardiac output as well as gastrointestinal blood flow. The maximal increase (×1.6±0.2; Fig. 4B) in $\dot{Q}_{cma}$ occurred at 30 min after the atropine injection, with the increase in $\dot{Q}$ reaching its respective peak (75±17%; Fig. 4A) after another 30 min. $\dot{Q}$ increased mainly by means of a large increase in stroke volume (66±18% at 1 h; Fig. 4D), enhanced by an initial (30 min) increase in f_H (from 57.5±1.9 beats min^{-1} to 63.2±1.3 beats min^{-1}; Fig. 4C).

Intrinsic (enteric) regulation of postprandial gut blood flow

To investigate the effects of removing the intrinsic (and the extrinsic) regulatory capacity of the gut by inhibiting the enteric innervation with the voltage-gated sodium channel inhibitor TTX (tetrodotoxin), we used three separate groups of fish that were kept under anesthesia on the surgical table during the entire experimental protocol. In untreated fish there was a significant decrease in the resistance of the gastrointestinal vasculature (25.0±4%), 30 min after the injection of the nutrient diet (Fig. 5A). This decrease in the vascular resistance of the splanchnic circulation in combination with a subsequent increase in cardiac output (25.0±9%; Fig. 6A) led to a substantial increase in gut blood flow (46.5±11%; Fig. 5B). There was no change in dorsal aortic pressure following any of the treatments (Fig. 6B). After an additional 30 min the respective values of $\dot{Q}_{cma}$ and $\dot{Q}$ had increased further, peaking at 159.4±15% and 127.4±8, respectively, while R_{cma} decreased by 28.5±6%. The initial (30 min) increase in $\dot{Q}$ was due to an elevation in both heart rate (from 39.4±3 beats min^{-1} to 47.1±5 beats min^{-1}; Fig. 6C) and stroke volume (15±6%; Fig. 6D). However, at 1 h f_H decreased, leaving V_S as the main contributor, with an increase of 15±4%.

The group of fish that had been pre-treated with TTX showed a fundamentally different response to that of the untreated group. There was no change in any of the measured variables and $\dot{Q}_{cma}$, $\dot{Q}$ as well as R_{cma} remained at their respective basal values for the entire 1 h after the injection of the predigested diet (Figs 5 and 6). The TTX treatment in itself had minor effects on the recorded variables except for $\dot{Q}_{cma}$, which decreased by 17.3±5% (data not shown). A control injection of saline had no significant effect on any of the measured variables.

DISCUSSION

This is, to our knowledge, the first study of how the extrinsic and intrinsic innervation of the gut influences the postprandial intestinal hyperemia in fish. The results indicate an importance of the extrinsic innervation in maintaining and regulating gut blood flow during normal conditions. However, it is probably of little importance to the gastrointestinal hyperemia after feeding. On the other hand, our results clearly show that the intrinsic innervation of the gastrointestinal tract, via enteric neurons, is ubiquitous and of fundamental importance to the postprandial hyperemia.

Regulation of nutrient-induced intestinal hyperemia

It is well known that the presence of digested or hydrolyzed food components in the intestine is the principal determinant of the postprandial hyperemia in mammals (Chou et al., 1978; Chou et al., 1985; Fara, 1984; Gallavan and Chou, 1985; Kvietys et al., 1980; Siregar and Chou, 1982; Sit et al., 1980) and in fish (Seth et al., 2009). The information concerning what controls this hyperemia is much more limited, especially the neural contribution. This is despite the fact that several studies have focused on the neural reflexes controlling the intestinal circulation in mammals as reviewed by, for example Vanner and Surprenant (Vanner and Surprenant, 1996).

Our results indicate that the intrinsic innervation of the gut is more important than extrinsic innervation in the control of postprandial hyperemia. This is reasonable since it seems that most of the submucosal arterioles are innervated directly by the enteric nervous system and the extrinsic innervation mainly innervates the larger superficial arteries and arterioles in fish (Olsson and Holmgren, 2001; Olsson and Holmgren, 2009; Olsson et al., 2009) and mammals (Furness, 2006). Extrinsic parasympathetic nerves do innervate the submucosal arterioles but only indirectly via enteric

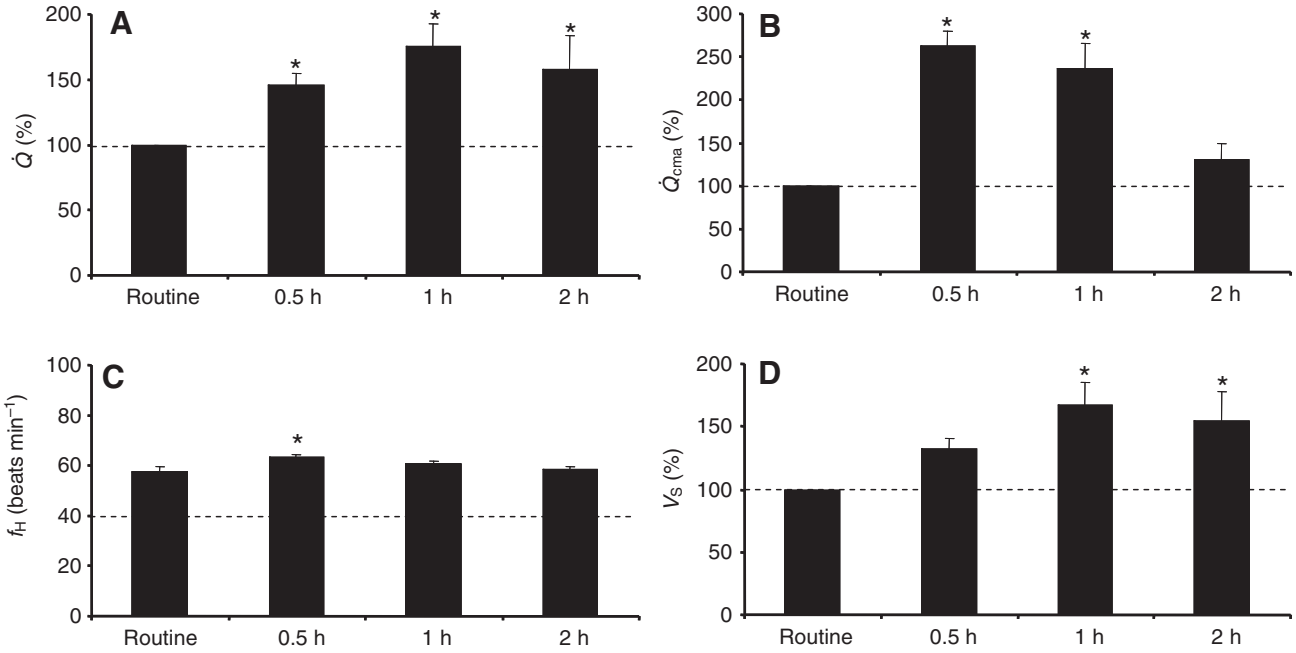

Fig. 4. *In vivo* measurements in unanaesthetized rainbow trout (*Oncorhynchus mykiss*) of (A) cardiac output ($\dot{Q}$) (B) dorsal aortic blood pressure ($\dot{Q}_{cma}$), (C) heart rate (f_H) and (D) stroke volume (V_S). Values are means ± s.e.m. Animals (N=4, 532±12 g) received an injection of atropine (1.2 mg kg^{-1}) into the proximal intestine. The dashed line indicates 100% for the normalized values and is arbitrarily set to 40 beats min^{-1} for f_H. *A significant difference from both the normal values and the control (P<0.05).

neurons, whereas some extrinsic sympathetic nerves innervate the submucosal arteriols directly (Holtzer, 2006).

Two of the main determinants of the postprandial hyperemia in mammals is almost certainly the oxygen tension (P_{O_2}) (Bohlen, 1980a; Bohlen, 1980b) and the increased sodium concentration (hyperosmolarity) in the tissue (Bohlen, 1982; Bohlen, 1998b; Chou et al., 1972). The decrease in oxygen tension as a result of the cost of nutrient uptake, enzyme secretion and assimilation as well as the subsequent increase in osmolarity could be sensed either in the mucosa and/or submucosa by some sort of chemoreceptor or in the venules by oxygen sensors such as hemoglobin (Dietrich et al., 2000; Ellsworth et al., 1995) or H_2S (Olson, 2008; Olson, 2009; Olson et al., 2008). However, there is a lack of knowledge about how a low P_{O_2} or the hyperosmolarity is communicated rapidly from the mucous to the submucosal arterioles where a large portion of any change in resistance and flow occurs (Gore and Bohlen, 1977), since the change in P_{O_2} or osmolarity is less profound in the submucosa than in the mucosa (Bohlen, 1998a).

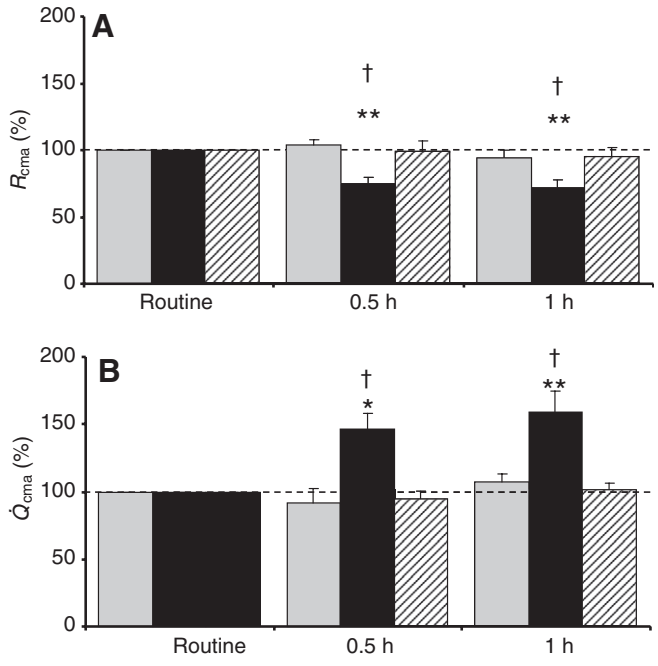

Fig. 5. *In vivo* measurements in anaesthetized rainbow trout (*Oncorhynchus mykiss*) of (A) vascular resistance of the coeliacomesenteric vascular system (R_{cma}) and (B) gastrointestinal blood flow at the coeliacomesenteric artery ($\dot{Q}_{cma}$). Values are means ± s.e.m. Control animals (N=8, 440±10 g) received an injection of saline (1.2 ml kg^{-1}) into the proximal intestine (grey bars). Untreated animals (N=8, 495±25 g) received an injection of a predigested diet (1.2 ml kg^{-1}) into the proximal intestine (black bars). Animals that had been pre-treated with tetrodotoxin (TTX; N=8, 445±9 g), a potent inhibitor of voltage-gated sodium channels, also received an injection of a predigested diet (1.2 ml kg^{-1}) into the proximal intestine (hatched bars). Asterisks denote a significant difference from both the normal values and the control (*P<0.05 and **0.01, respectively). †A significant difference between the untreated and the denervated group (P<0.05).

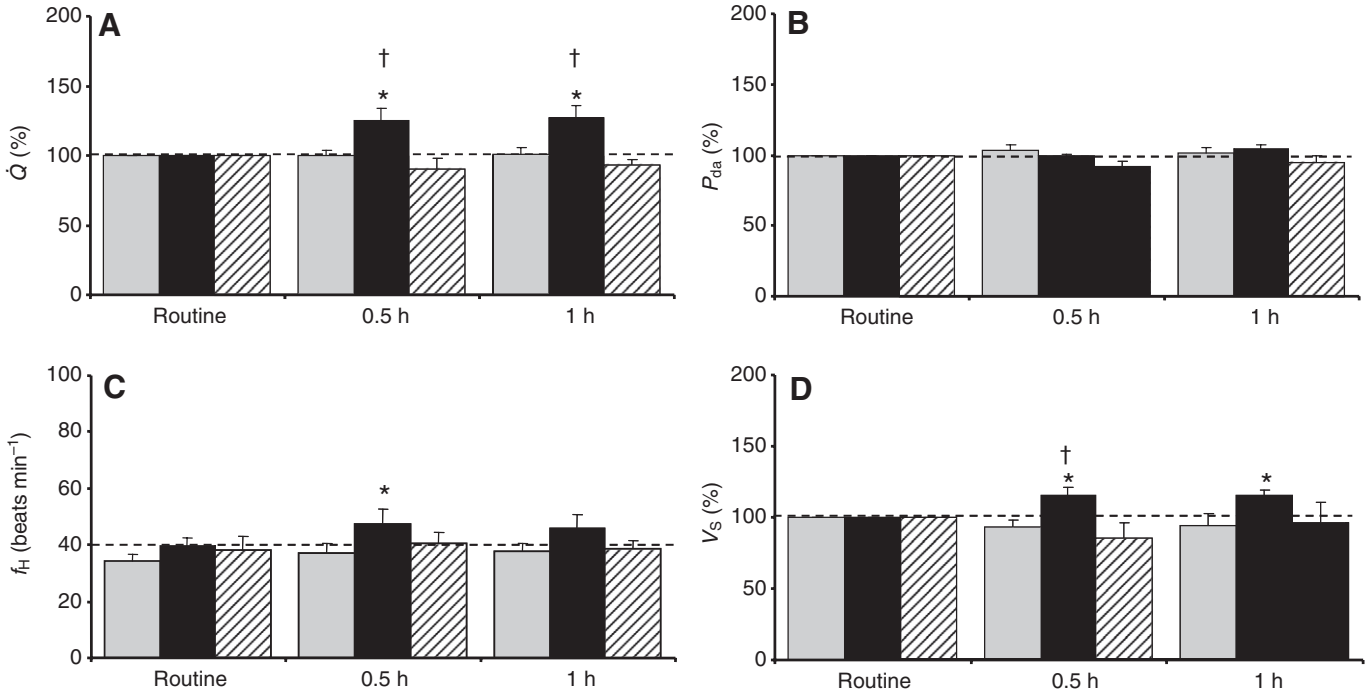

Fig. 6. *In vivo* measurements in anaesthetized rainbow trout (*Oncorhynchus mykiss*) of (A) cardiac output ($\dot{Q}$), (B) dorsal aortic blood pressure (P_{da}), (C) heart rate (f_H) and (D) stroke volume (V_S). Values are means ± s.e.m. Control animals (N=8, 440±10 g) received an injection of saline (1.2 ml kg^{-1}) into the proximal intestine (grey bars). Untreated animals (N=8, 495±25 g) received an injection of a predigested diet (1.2 ml kg^{-1}) into the proximal intestine (black bars). Animals which had been pre-treated with tetrodotoxin (TTX; N=8, 445±9 g), a potent inhibitor of voltage-gated sodium channels, also received an injection of a predigested diet (1.2 ml kg^{-1}) into the proximal intestine (hatched bars). The dashed line indicates 100% for the normalized values and is arbitrarily set to 40 beats min^{-1} for f_H. *A significant difference from both the normal values and the control (P<0.05); †a significant difference between the untreated and the denervated group (P<0.05).

We, therefore, suggest that the signal is relayed from the mucosa, where the vascular effects could be both direct and indirect, *via* enteric nerves from the myenteric plexuses of fish. The signal is subsequently sent to the submucosal vasculature, *via* nitrergic perivascular nerves releasing vasoactive factors such as, nitric oxide (NO) (Jennings et al., 2004), endothelial-dependent prostaglandins (Kagstrom and Holmgren, 1997), neuropeptide Y (Shahbazi et al., 2002) and vasoactive intestinal polypetide (Jensen et al., 1991; Kagstrom and Holmgren, 1997). In mammals there is also a muscarinic-receptor-mediated release of endothelial NO (Vanner et al., 1993). However, given that it is still debatable whether or not NO is synthesized and released only from nerves in fish or if there is also an endothelial subform (Olson and Donald, 2009; Olson and Villa, 1991), prostaglandins could function as an endothelially derived vasorelaxing factor in fish, comparable to that of NO in mammals (Jennings et al., 2004; Shahbazi et al., 2002).

Even though the mechanism by which the enteric nervous system controls the postprandial hyperemia in fish is unknown, it must be of fundamental importance given that response was completely abolished when blocking the enteric nervous system. A similar importance or involvement of the enteric nervous system has been shown in several mammalian studies as well (Chou et al., 1972; Neild et al., 1990; Surprenant, 1994). On the other hand, other studies show little importance of neural control mechanisms, whether extrinsic or intrinsic (Nyhof and Chou, 1983; Nyhof et al., 1985). The reason for this difference is not clear, but could depend on species differences and the stimulus used to evoke the hyperemia. For example, in vessel structures where the arterioles and the venules lie in close proximity the signal could perhaps diffuse from the venule to the arteriole (Bohlen, 1998b) or the signal could propagate as a series of events within the vascular wall (Collins et al., 1998; Ellsworth et al., 2009).

Other studies indicate that mechanical stimuli to the intestinal mucosa induce a TTX-sensitive vasodilatory reflex intrinsic to the gut (Vanner, 1993). However, it is unclear whether this vasodilatory reflex is primary or secondary to a change in gut motility, although changes in gut motility are usually associated with a subsequent rearrangement in blood flow within the gut, with only minor effects on the net flow (Chou, 1982; Chou and Gallavan, 1982; Chou and Grassmick, 1978). TTX could perhaps also have interfered with the nutrient uptake in the intestine, but dibucaine (an amide local anesthetic) not TTX is generally used to block intestinal nutrient uptake (Nyhof and Chou, 1983). Certain types of chemical stimuli, such as capsaicin, induce an extrinsically mediated vasodilation as it can be blocked with extrinsic nerve sectioning (Vanner, 1994; Vanner and Bolton, 1996), but most studies show little importance of extrinsic innervation in maintaining a postprandial intestinal hyperemia (Nyhof and Chou, 1981; Nyhof and Chou, 1983; Nyhof et al., 1985; Takagi et al., 1988; Vanner and Surprenant, 1996; Vatner et al., 1970). By contrast, the increase in gastric blood flow observed in dogs during feeding is most probably mediated *via* a vagal reflex pathway, as it can be blocked with topical administration of local anesthetic to the vagi (Takagi et al., 1988). This suggests that the gastric and the intestinal hyperemia are not equally controlled, but few studies have focused on the reflex pathways that are elicited by physiological stimuli during feeding.

In order to study a possible involvement of an acetylcholine-mediated release of NO, or any related compound, we pretreated

animals with atropine. However, the injection of atropine produced a rather unexpected response as it caused a substantial increase in blood flow, which was larger than any postprandial change in blood flow. This effect could be to a parasympathetic vasoconstrictor tonus on the gastrointestinal vasculature, which is unlikely considering that the parasympathetic nervous system imposes a strong vasodilator tone in the gastrointestinal tract, at least in mammals (Holzer, 2006). It could also indicate that there is a change in the tonus of the smooth musculature of the gastrointestinal tract leading to a change in the transmural pressure of the vasculature. This is also unlikely considering that there is a considerable vasorelaxation in isolated gastrointestinal vessels when adding atropine (Seth et al., 2010). The most likely explanation is that the effect instead represents a pharmacological artifact. There are a few reports in mammals of a non-cholinergic, non-adrenergic vascular effect of atropine, possibly mediated *via* K^+ channels, at least in adrenergically stimulated vessels (Liu et al., 2004). This calls for caution when interpreting the results using atropine in fish as well as mammals.

Regulation of basal intestinal blood flow

Our results clearly show that whereas extrinsic nerves (i.e. the paired vagus and the splanchnic nerve) are of minor importance in the control of the intestinal postprandial hyperemia the splanchnic nerve maintains and regulates the basal tone or resistance of the intestinal vasculature. This is in agreement with other studies in fish, showing an adrenergic vasoconstrictor mechanism in the red Irish lord (*Hemilepidotus hemilepidotus*) (Axelsson et al., 2000), sea raven (*Hemitripterus americanus*) (Axelsson et al., 1989) and the Atlantic cod (*Gadus morhua*) (Axelsson and Fritsche, 1991). Similarly, in mammals there is little evidence of a vasoconstrictor mechanism intrinsic to the gastrointestinal tract (Surprenant, 1994) and extrinsic mechanisms are thus prevalent (Rothe, 1984). The extrinsic innervation is probably important in controlling the distribution of blood within the entire animal, e.g. during exercise, or other situations where there is a high demand on the cardiovascular system. However, it is at present unknown to what extent neurally released catecholamines contribute compared with circulating catecholamines in fish (Axelsson et al., 2000; Olsson and Holmgren, 2009). Circulating catecholamines are less likely to be important since plasma levels are generally too low to explain a resting adrenergic tonus on the vasculature and the heart in the Atlantic cod (Axelsson, 1988; Axelsson et al., 1987; Axelsson and Nilsson, 1986). Furthermore, during exercise there can be an increase in the tonus of the gastrointestinal vasculature without a subsequent increase in the plasma levels of circulating catecholamines, indicating a neurally mediated adrenergic tonus (Farrell et al., 2001), as we have also shown here.

It is also, at present, unknown why cutting the extrinsic nerves leads to a significant decrease in the resistance of the innervated blood vessels, whereas the TTX treatment, which abolishes the function of both the extrinsic and the intrinsic nerves, has a very limited effect on the resistance of the very same vessels. The presence of TTX-insensitive autoregulatory mechanisms in combination with the anesthesia could contribute (Mellander et al., 1987), and also the fact that the gastrointestinal tract seems poorly autoregulated, at least in mammals (Granger et al., 1982). Another possible explanation is that the denervation of the gastrointestinal tract leads to a subsequent increase in the resistance of the remaining vasculature (Kremer and Wright, 1932). This would maintain the systemic blood pressure while at the same time increasing the gastrointestinal blood flow, through a redistribution of the available blood, as long as the baroreceptor innervation is left intact. Furthermore our results indicate that a muscarinic receptor-mediated mechanism could be involved in the control of the basal vascular resistance, although as stated above, these results should be interpreted with caution, especially given that atropine also possibly acts via a non-muscarinic mechanism.

Conclusions and future perspectives

In conclusion, we show that the intrinsic innervation of the intestine *via* the enteric nervous system is of fundamental importance in inducing and maintaining a postprandial intestinal hyperemia in rainbow trout. The exact mechanism by which this occurs warrants further investigations, and it is at present unknown what part of the enteric nervous system is essential to the hyperemia. It could be that the effect is secondary to the lack of nutrient uptake or gut motility although this is less likely considering the reasons discussed above. It is possible that a cholinergic limb is involved, and our results using the muscarinc receptor antagonist atropine, indicate that muscarinic receptors might be involved in the control of the vasculature at least under normal conditions, although this is unlikely.

More knowledge on the transmitters available and their respective functions, and the anatomy and dispersion of these neurons is also essential and could most probably be obtained through a combination of *in vivo* measurements and immunohistochemistry. In fish it is not known, for example, to what extent precapillary sphincters contribute to changes in the vascular resistance of the gastrointestinal tract. We also show that although the extrinsic innervation by the paired vagus and the splanchnic nerve is of little importance to the hyperemia it still controls and maintains the basal tonus of the intestinal vasculature.

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LIST OF SYMBOLS AND ABBREVIATIONS

f_H	heart rate
P_{da}	dorsal aortic blood pressure
P_{ven}	central venous blood pressure
$\dot{Q}$	cardiac output
$\dot{Q}_{cma}$	gastrointestinal blood flow
R_{cma}	resistance of the gastrointestinal vasculature
TTX	tetrodotoxin
V_S	stroke volume

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