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Baroreflex mediated control of heart rate and vascular capacitance in trout

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Summary

The baroreflex was triggered by altering branchial blood pressure with pre- and post-branchial occlusions for 30 s in rainbow trout *Oncorhynchus mykiss*. The cardiac limb of the baroreflex was monitored by continuous heart rate (f_H) measurements. Responses of venous capacitance vessels were assessed, immediately following either occlusion, by measuring mean circulatory filling pressure (MCFP). Arterial responses were evaluated as the change in dorsal aortic blood pressure (P_{da}) before and after pre-branchial occlusion. In untreated fish pre-branchial occlusion resulted in tachycardia (62.4±2.4 to 69.1±1.7 beats min^{-1}), decreased venous capacitance reflected as an increase in MCFP (0.17±0.03 to 0.27±0.03 kPa) and increased P_{da} (4.0±0.2 kPa compared to 3.2±0.1 kPa before occlusion). Post-branchial occlusion somewhat reversed the responses since f_H decreased (62.4±2.4 to 53.0±3.1 beats min^{-1}), whereas MCFP remained unaltered. Treatment with the α-adrenergic blocker prazosin (1 mg kg^{-1}) increased resting MCFP to 0.33±0.03 kPa and appeared to abolish both venous and arterial responses to branchial occlusion. Subsequent atropine treatment (1.2 mg kg^{-1}) abolished all chronotropic responses. We present for the first time ample evidence for baroreflex-mediated control of cardiovascular homeostasis, including both the chronotropic and the vascular limb of the baroreflex in an unanaesthetized fish. Furthermore, a novel technique to cannulate and occlude the dorsal aorta, using a Fogarty thru-lumen embolectomy catheter, is explained.

Key words: baroreflex, bradycardia, embolectomy catheter, MCFP, tachycardia, teleost, vascular resistance, venous capacitance, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Mammalian systemic baroreceptors, which are located in the aortic arch and the carotid sinuses, provide beat-to-beat afferent information to the central nervous system to maintain cardiovascular homeostasis. The primary reflex response to a drop in arterial blood pressure, for example due to an orthostatic change, is increased cardiac output mediated by decreased cholinergic and increased adrenergic tone on the heart, which increases heart rate and possibly stroke volume. Associated with the tachycardia is an increased peripheral resistance, due to an increased sympathetic outflow to arteriolar resistance vessels. Furthermore, sympathetic stimulation of the venous vasculature decreases venous capacitance, which mobilizes hemodynamically inactive blood from the unstressed vascular compartment into the hemodynamically active stressed vascular compartment. This is reflected as an increase in the mean circulatory filling pressure (MCFP), which consequently increases the pressure gradient for venous return (Pang, 2001).

In teleosts, evidence for a cardiovascular baroreflex is circumstantial and typically stems from secondary observations made during pharmacological studies. Early workers noticed that injections of adrenaline, which caused a rapid increase in arterial pressure, also elicited a transient bradycardia that could be blocked with atropine (Helgason and Nilsson, 1973; Randall and Stevens, 1967; Stevens et al., 1972; Wood and Shelton, 1980). Owing to the fact that vascular resistance was pharmacologically manipulated in these closed-loop studies, conclusions about the vascular limb of the baroreflex response could not readily be made. Few studies have previously dealt with the baroreflex in fish, using open-loop techniques that include both vascular and cardiac responses (see West and Van Vliet, 1994, for an explanation on open- *vs* closed-loop studies). Farrell (1986) used a technique where a neoprene collar was placed around the head region of unanaesthetized, but restrained sea ravens *Hemitripterus americanus*, thus separating the trunk from the head region. Elevation of the water level above the tail region induced a rapidly developing bradycardia that could be blocked with atropine. After returning to the initial water level, a decrease in systemic resistance associated with a drop in arterial blood pressure was frequently observed. The smooth muscle relaxant Papaverine did not seem to alter this response and the author explained the phenomenon as pooling of blood as the vessels resumed their initial diameter.

The precise location(s) of baroreceptors in the circulatory system in fish remains to be substantiated (Nilsson and Sundin,

1998). Using an open-loop system, it was shown in the anaesthetized carp *Cyprinus carpio* that an elevation of pressure in the intrabranchial afferent arteries resulted in a drop in heart rate and arterial blood pressure (Ristori, 1970; Ristori and Dessaux, 1970). Mott (1951) obtained the same results using a similar experimental preparation in the eel *Anguilla anguilla*. These early observations pointed out the gills as the primary site for baroreceptor sensitivity in fish, a fact that still seems to be the general consensus (Nilsson and Sundin, 1998).

As previously pointed out, it is well established in mammals that a drop in arterial blood pressure also results in an increased sympathetic outflow to venous capacitance vessels. This leads to mobilization of venous blood and an increased pressure gradient for venous return. Despite the fact that an active regulation of venous capacitance is present in fish (Conklin et al., 1997; Olson et al., 1997; Zhang et al., 1998), there is no information on the baroreflex control of venous capacitance in fish.

In the present study a non-pharmacological open-loop technique was used to study the cardiac and the vascular limb of the baroreflex in unanaesthetized trout. Continuous measurements of dorsal aortic blood pressure, venous blood pressure and heart rate were conducted during 30 s of post-branchial dorsal aortic occlusion (increased branchial blood pressure) and ventral aortic pre-branchial occlusion (decreased branchial blood pressure). To evaluate the baroreflex control of venous capacitance, mean circulatory filling pressure was measured during zero-flow conditions, immediately following either occlusion.

Material and methods

Animals

Rainbow trout *Oncorhynchus mykiss* Walbaum of mixed sexes in the size range of 650–940 g were used. The fish, purchased from a local fish farm, were kept in 2 m^3 tanks with circulating water at 15°C supplied by the departmental water system. Photoperiod was adjusted to natural conditions and the animals were fed a maintenance diet of commercial trout pellets. An acclimation period of at least 1 week for newly arrived fish was always applied, before any experiments were conducted. Ethical permits covered all experiments (96/2001).

Instrumentation and surgical procedures

Preliminary studies indicated that post-surgical viability increased substantially when the instrumentation of the fish was broken up in two steps, with a short (1–2 h) recovery period after the first step. Fish that displayed irregular or abnormal behaviour after recovery were not used for further instrumentation. The fish were instrumented as detailed below (Fig. 1).

Dorsal aortic cannulation

Fish were randomly collected from the holding tanks and anaesthetized in MS-222 solution (150 mg l^{-1}) buffered with sodium bicarbonate (300 mg l^{-1}). Prior to surgery the fish were weighed and transferred to an operating table that was covered with water soaked foam rubber. During surgery a recirculating system at 10°C continuously irrigated the gills with water containing sodium bicarbonate-buffered MS-222 (150 mg l^{-1} and 75 mg l^{-1}, respectively).

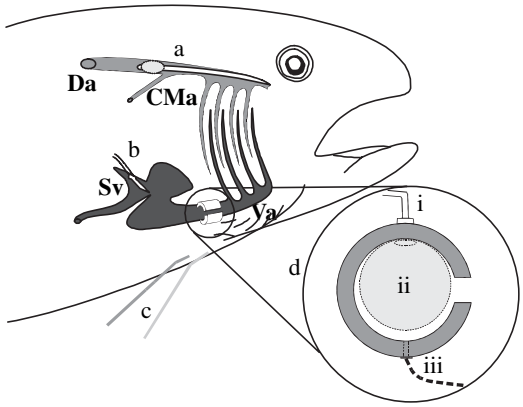

Fig. 1. Schematic drawing of trout instrumented with (a) occlusion catheter in dorsal aorta (Da), positioned distal to celiacomesenteric artery (CMa); (b) catheter in sinus venosus (Sv); (c) percutaneous ECG-electrodes and (d) occlusion probe around ventral aorta (Va). A cross-section of the ventral aortic probe is magnified to illustrate (i) PE-50 catheter connected to latex-balloon; (ii) inflatable latex-balloon and (iii) doppler crystal with lead.

To measure systemic arterial blood pressure the dorsal aorta was cannulated using a heparinized Fogarty thru-lumen embolectomy catheter (model 12TLW803F, Edwards Lifesciences, Irvine, CA, USA) with an outer diameter of 1 mm and a length of 80 cm. The catheter has two lumens, one being connected to an inflatable latex balloon. The second lumen served to give the arterial blood pressure.

A slightly modified method to cannulate the dorsal aorta *via* the roof of the buccal cavity by means of a guide wire was used (Axelsson and Fritsche, 1994). Approximately 10 mm of tapered PE-50 tubing was fitted onto the tip of the embolectomy catheter. This gave the catheter a smooth tapered shape and a good fit to the guide wire, thus facilitating the cannulation procedure. A mark was made 50 mm from the anterior tip of the embolectomy catheter. The catheter was advanced into the vessel until the mark was at the level of the entry point, located at the first pair of gill arches. This served to give an indication of how far down the length of the dorsal aorta the inflatable balloon was positioned. This position was below the bifurcation point of the coelomesenteric artery. The cannulation procedure occasionally caused some initial hemorrhage, which was stopped by gently pressing on the wound until the bleeding ceased spontaneously within a few minutes. A bolus of approximately 1 ml of heparinized (100 IU ml^{-1}) saline (0.9%) was administered following cannulation. The catheter was attached with one suture, close to the first pair of gill arches. In order to protect the catheter from mechanical damage from the teeth, the first 10 cm of the catheter protruding from the tissue was covered with PE-200 tubing. The catheter was bent at 180° inside the buccal cavity

and exteriorized under the right operculum. Three sutures were used to attach the catheter to the roof of the buccal cavity along the row of teeth. An additional suture in the skin was placed posterior to the operculum. The entire dorsal aortic cannulation procedure typically took around 20 min. Subsequently the fish were allowed to recover in covered tanks connected to the departmental water system.

Cannulation of Sinus venosus

Central venous blood pressure was recorded from the Sinus venosus, which was non-occlusively cannulated. The fish was positioned on its right side. The left operculum and the gills were carefully retracted and a less than 10 mm incision, running approximately 45° dorsoventrally was made. Starting point was on top of the cleithrum, ending posterior to the Vth gill-free branchial arch. The lateral part of the Ductus of Cuvier was dissected free using blunt dissection. The vessel was gently pulled, ideally ~5 mm, and secured with a 4-0 suture. A small hole was cut in the upper part of the venous tissue and a heat-bubbled PE-50 catheter was inserted. The catheter was directed towards the heart and inserted approximately 10 mm into the sinus. The catheter was secured in the sinus with a 4-0 suture above the bubble and to the skin with two additional sutures close to the opercular opening. For further details, see Altimiras and Axelsson (2004).

Attachment of ventral aortic probe

An occlusion probe (i.d. ~1.8 mm), custom-made from Perspex, was placed around the ventral aorta (see Fig. 1 for details). The cuff-type probe was equipped with a vascular occlusion rubber balloon. This was constructed from approximately 1 m of heat-flared PE-50 catheter that was bent at a right angle approximately 5 mm posterior to the flared end. The catheter was filled with water and a small piece of dental latex rubber (model Thin, Dental Dam, Coltène/Whaledent Inc, USA and Canada) was tied with a 4-0 suture around the flared end. A small hole was drilled perpendicular to the lumen, using an injection needle (20 G). Using a round-type dental drill the luminal side of the hole was countersunk to fit the flared end of the catheter. The free end of the catheter was pulled through the hole from the luminal side. The flared end was positioned with the latex rubber facing the lumen, where it was locked from the outside with a ~3 mm piece of heat-flared PE-90 tubing. Inflation of the latex rubber with a syringe resulted in a bubble developing inside the probe lumen, thus occluding the ventral aorta. On the opposite side of the occlusion device, another similar hole was drilled. A 20 MHz Doppler flow crystal (Iowa Doppler products, Iowa City, IA, USA) was glued to the probe. The flow recordings were only used to help indicate when zero-flow was reached during the occlusion manoeuvre.

The probe was positioned around the ventral aorta by making an incision on the right side of the isthmus where the aorta was exposed by blunt dissection. A suture was placed around the vessel that was carefully lifted to facilitate placement of the probe. Two lateral sutures collectively secured the Doppler crystal lead and the occlusion catheter to the skin posterior to the operculum.

Attachment of ECG-electrodes

To enable continuous measurements of heart rate also during zero flow conditions, two custom-made ECG-electrodes were placed percutaneously close to the heart. Electrodes were made from Grass Stimulatory electrodes (Grass Instruments, Quincy, MA, USA), modified by cutting the original ending and replaced with around 30 mm of platinum wire soldered to the bare ends. To position the electrodes a 0.8 mm injection needle was used to penetrate the skin approximately 10 mm anterior to the pectoral fins. The platinum wires were positioned under the skin on either side of the heart and secured with skin sutures. Finally all leads and catheters were collectively attached to the back of the fish with a common suture.

After surgery the fish was transferred to a holding chamber, or immediately to the experimental chamber, both connected to the departmental water system. The fish were placed in the experimental chamber at least 24 h prior to experiments. To minimize stress from visual stimuli, all chambers were thoroughly covered with non-transparent black plastic. Experiments were performed 24–72 h following surgery.

Experimental protocol

To stimulate branchial baroreceptors with elevated blood pressure the embolectomy catheter latex balloon, located in the dorsal aorta (post-branchial occlusion), was inflated for 30 s with approximately 0.5 ml of air. This gave the inflated balloon an approximate diameter of 6 mm. *Post mortem* analysis in combination with the non-pulsatile pattern of the P_{da} recording during occlusion (see Figs 3 and 4), revealed that this was sufficient to occlude the post-branchial portion of the dorsal aorta and hence, increase the branchial blood pressure. Low branchial blood pressure was initiated for 30 s by occluding the ventral aorta (pre-branchial occlusion) by means of the ventral aortic occluder. Complete occlusion, i.e. zero-flow, was attained by inflating the latex bubble and simultaneously observing when the ventral aortic flow and the dorsal aortic pressure dropped (Figs 3 and 4). An occlusion period as long as 30 s was chosen based on the findings of Zhang et al. (1995), who noticed that venous reflex responses to cardiac fibrillation in the trout first appeared after around 10 s. Although this is considerably less than the 2 min of vascular compression used in the sea raven (Farrell, 1986), preliminary studies revealed that this stimulation was enough to trigger solid cardiac as well as vascular responses. Heart rate (f_H), dorsal aortic pressure (P_{da}) and central venous pressure (P_{ven}) were continuously recorded. Mean circulatory filling pressure was measured 2 s following pre- or post-branchial occlusion assuming that most of the venous response to pre- or post-branchial occlusion still remained, or during control conditions without manipulated branchial blood pressure. The order of the three manipulations of branchial blood pressure was randomized and separated by 30 min. To measure MCFP the ventral aortic occlusion technique, previously described for trout (Zhang et al., 1998),

was employed. Zero-flow conditions were obtained by inflating the latex bubble in the ventral aortic probe for 8 s. This period is generally assumed to be too short to induce any reflex responses, but long enough to achieve a stable venous plateau pressure (Zhang et al., 1995). During ventral aortic occlusion systemic arterial blood pressure dropped instantaneously, whereas venous blood pressure increased. MCFP was subsequently calculated as the average of 2 s of the venous plateau pressure period, taken between 5 and 7 s of zero flow.

The same protocol was repeated 1.5–2 h after α-adrenergic blockade with prazosin (1 mg kg^{-1}, Pfizer, Sandwich, England) and, subsequently, 30 min after an additional atropine treatment (1.2 mg kg^{-1}, Sigma, St Louis, MO, USA). Drugs were dissolved in physiological saline (0.9%, 1 ml kg^{-1}) and administered slowly *via* the venous catheter. Sham injections with physiological saline (0.9%, 1 ml kg^{-1}) were also done in identical experiments as previously described.

Equipment and data acquisition

Arterial and venous blood pressures were measured using pressure transducers (model DPT-6100, pvb medizintechnik, Kirchseeon, Germany) connected to a 4ChAmp amplifier (Somedic, Hörby, Sweden). The equipment was calibrated against a static water column, with the water surface of the experimental chamber serving as baseline. Cardiac output was recorded using a directional-pulsed Doppler flow meter (model 545C-4, University of Iowa, Iowa City, IA, USA) connected to the Doppler crystal in the ventral aortic flow probe. A Grass amplifier (model 7P511K, Grass Instruments, USA) amplified the ECG-signal and triggered a Tachograph Recorder unit (model 7P44D, Grass Instruments, Quincy, MA, USA) in order to obtain heart rate. Data were digitally stored on a PC running a custom made program, General Acquisition (Labview version 6.01, National Instruments, Austin, TX, USA).

Statistical analysis and calculations:

The heart rate response during ventral or dorsal aortic occlusion was calculated as the average value of the last 20 s of each occlusion. Mean values of 120 s before ventral and dorsal aortic occlusion were pooled to serve as an unstimulated control. For MCFP measurements, the average value of P_{ven} between 5–7 s of a short ventral aortic occlusion was always used (Zhang et al., 1998). The effect of altered branchial blood pressure or the effects of pharmacological treatment were evaluated statistically using a two-tailed Wilcoxon matched-pairs signed-ranks test, with a fiduciary level of 0.05. When multiple comparisons were made a modified Bonferroni-test was applied (Holm, 1979).

Results

Chronotropic responses to changes in branchial blood pressure

Heart rate (f_H) was 62.4±2.4 beats min^{-1} in untreated control fish. Heart rate was unaffected by prazosin treatment (62.6±2.3), but increased significantly after a subsequent injection with atropine (69.1±2.1) (Fig. 2A). In control fish post-branchial occlusion caused significant bradycardia (53.0±3.1). Conversely, pre-branchial occlusion caused a significant tachycardia (69.1±1.7). Prazosin treatment attenuated the bradycardia (57.8±2.9) but not the tachycardia (68.8±2.3). Atropine treatment completely abolished both responses (Fig. 2A).

Changes in vascular capacitance in response to altered branchial blood pressure

Control fish had a routine MCFP of 0.17±0.03 kPa, which increased significantly to 0.33±0.03 kPa following prazosin treatment and remained elevated after additional atropine treatment (0.34±0.04 kPa) (Fig. 2B).

Following pre-branchial occlusion MCFP in the untreated fish, increased significantly to 0.27±0.03 kPa compared with routine MCFP in control fish. Blockade of α-adrenergic receptors abolished this response since mean circulatory filling pressure remained unchanged with pre-branchial occlusion after prazosin as well as prazosin+atropine treatment. However post-branchial occlusion in the control fish did not significantly alter MCFP (0.19±0.02 kPa) to routine MCFP. Instead, post-branchial occlusion significantly decreased MCFP after treatment with prazosin (from

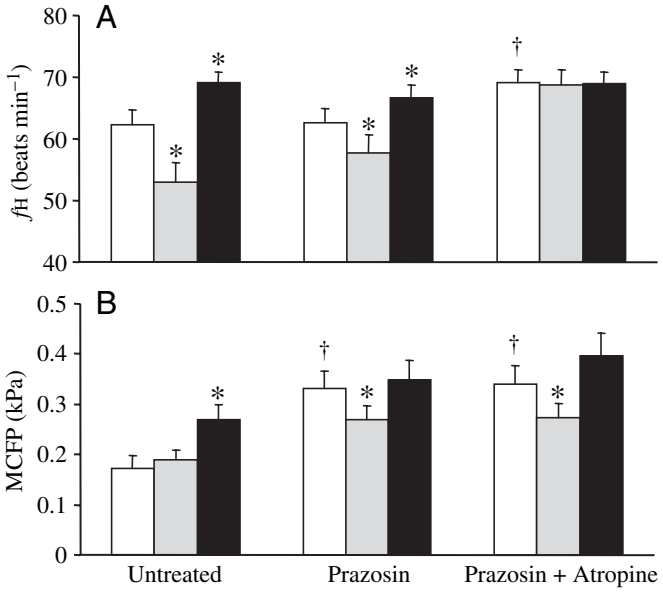

Fig. 2. Heart rate (A; f_H) and mean circulatory filling pressure (B; MCFP) in untreated, prazosin-treated (1 mg kg^{-1}) and atropine+prazosin treated trout (1 mg kg^{-1} and 1.2 mg kg^{-1}, respectively) during control conditions (white bars), post- (grey bars) and pre-branchial occlusion (black bars). Control heart rate represents pooled mean values + S.E.M. of 120 s prior to occlusion. Heart rate during post- and pre-branchial occlusion are mean values + S.E.M. of the last 20 s of the 30 s occlusion. MCFP is measured in unstimulated fish and immediately following 30 s of stimulation with post- or pre-branchial occlusion. Statistically significant difference *from the control within each treatment and †for control values between treatments ($P \leqslant 0.05$).

Table 1. *Effects of repeated pre- and post-branchial occlusions and sham-injections in rainbow trout* Oncorhynchus mykiss

	Untreated				Sham I				Sham II			
	N	Control	Post	Pre	*N*	Control	Post	Pre	*N*	Control	Post	Pre
f_H (beats min^{-1})	9–10	58.2±3.5	43.5±3.7*	80.7±4.1*	7–9	61.8±3.7†	47.9±4.4*	76.2±4.2*	7–9	66.5±3.3†	55.8±3.8*	79.2±4.7*
MCFP (kPa)	9	0.12±0.03	0.13±0.04	0.23±0.04*	8	0.19±0.04†	0.20±0.04	0.29±0.04*	7–8	0.20±0.03†	0.23±0.04	0.31±0.05*

Values are means ± S.E.M. of heart rate (f_H) and mean circulatory filling pressure (MCFP) in unstimulated control, post- and pre-branchial occlusion.

A sham-injection of physiological saline (1 ml kg^{-1}), following the same experimental protocol as in Fig. 2, was administered.

*Statistically significant difference from the control ($P \leq 0.05$) within each treatment, †statistically significant difference between control values ($P \leq 0.05$).

0.33±0.03 kPa to 0.27±0.03 kPa), a response that was unchanged with further treatment with atropine (from 0.34±0.04 kPa to 0.27±0.03 kPa).

Systemic arterial responses to decreases in branchial blood pressure

Arterial responses to pre-branchial occlusion were evaluated by comparing the difference in P_{da} before and immediately after a ventral aortic occlusion in control and prazosin-treated fish (Fig. 3).

The average value of P_{da} in untreated fish after pre-branchial occlusion was significantly higher (4.0±0.2 kPa) compared to the average value before occlusion (3.2±0.1 kPa). Blockade of α-adrenoceptors with prazosin reversed the response and left P_{da} significantly reduced following pre-branchial occlusion (2.3±0.1kPa compared to 2.6±0.2kPa in the control).

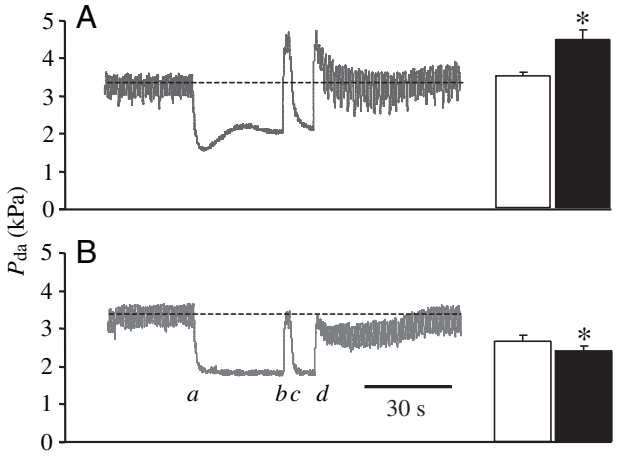

Fig. 3. Representative original recordings of dorsal aortic blood pressure (P_{da}) from one trout before treatment (A) and after prazosin (1 mg kg^{-1}) treatment (B). 30 s of pre-branchial occlusion starts at *a* and stops at *b* followed by a mean circulatory filling pressure manoeuvre between *c* and *d*. White bars represent 10 s mean values + S.E.M. of P_{da} prior to occlusion and black bars represent 10 s mean values + S.E.M. of P_{da} immediately following the mean circulatory filling pressure manoeuvre. *Statistically significant difference ($P \leq 0.05$) between values. Note the reflex increase in pressure during occlusion and the following increase in pressure in the untreated fish. After prazosin treatment this reflex response is absent.

Sham experiments

Despite qualitatively equal responses, sham experiments revealed a slight increase in control values of MCFP and f_H after repeated occlusions (Table 1). The underlying mechanism to this is unknown, but a host of potential neuro-endocrine substances could be released during the course of the experiment. Substances such as arginine vasotocin (Conklin et al., 1997); chatecholamines (Zhang et al., 1998), endothelin (Hoagland et al., 2000) and neuropeptides (Olson et al., 1997) are all known to affect the cardiovascular (including venous) system in fish. Regarding these technical limitations, normalized gain for the baroreflex was not calculated. Hence, the following discussion on the cardiovascular responses to altered branchial pressure and the effects of pharmacological treatment should be seen in a qualitative context, rather than in absolute quantitative terms.

Discussion

Chronotropic responses to changes in branchial blood pressure

Few studies have previously investigated baroreflex mediated control of heart rate in teleosts by means of non-pharmacological open-loop techniques (i.e. Farrell, 1986; Mott, 1951; Ristori, 1970; Ristori and Dessaux, 1970). As far as we know, the present experiment is the first where baroreceptors have been both unloaded and stimulated in a conscious teleost. Our findings strongly support the view that branchial blood pressure is inversely related to heart rate, as a means of controlling cardiovascular homeostasis on a beat-to-beat basis. The changes in heart rate induced by both pre- and post-branchial occlusion were fast and generally commenced within seconds (Fig. 4). This is indicative of a truly reflex mediated response and not due to humoral factors released by myocardial or vascular stretch, for instance. The bradycardia during dorsal aortic occlusion typically lasted throughout the entire episode of occlusion, indicating that the response was not a startle response, and as such would have been expected to be shorter in duration and possibly associated with struggling (Farrell, 1986). Furthermore, based on the significant tachycardia observed after ventral aortic occlusion, an argument could be made that manipulation of branchial blood pressure *per se*, does not induce a startle response.

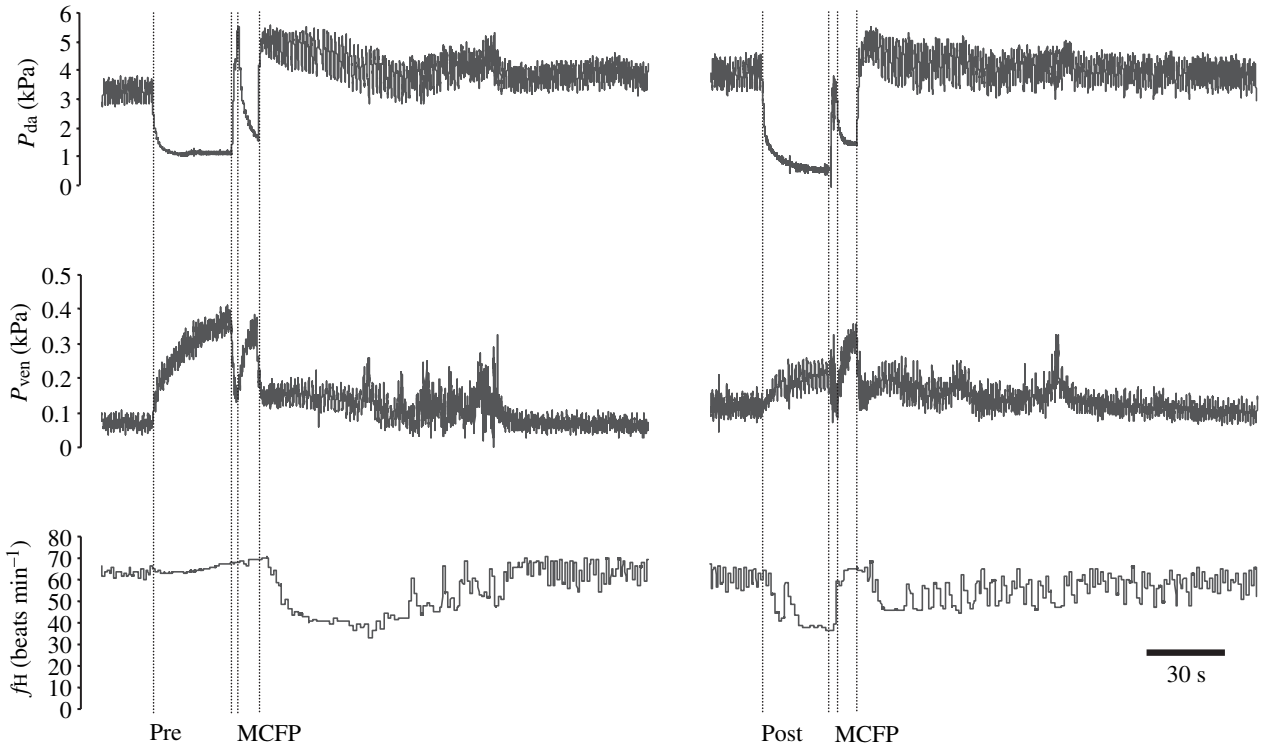

Fig. 4. Representative original recordings of heart rate (f_H), dorsal aortic pressure (P_{da}) and venous pressure (P_{ven}) from an untreated trout. Left-hand panels demonstrate a pre-branchial occlusion (Pre) and right-hand panels demonstrate a post-branchial occlusion (Post). Both occlusions are followed by a mean circulatory filling pressure manoeuvre (MCFP).

The complete lack of chronotropic responses to altered branchial blood pressure after atropine treatment (Fig. 2A), further supports the reflex origin of the response and shows that baroreflex mediated modulation of heart rate is exclusively cholinergic. The same conclusion has previously been drawn in other teleosts such as the eel (Mott, 1951), the carp (Ristori, 1970), and the sea raven (Farrell, 1986).

Vascular responses to changes in branchial blood pressure

In untreated animals MCFP was significantly higher when it was preceded by a pre-branchial occlusion compared to the control (Fig. 2B). We argue that the drop in branchial blood pressure during the occlusion was detected by baroreceptor afferents that initiated a reflex stimulation of capacitance vessels. The complete block of the vascular response to pre-branchial occlusion by prazosin identifies the α-adrenergic system as the major mediator of reflex control of vascular capacitance in fish.

Capacitance of the vascular system is dependent on smooth muscle tone and vascular compliance and represents the relationship between contained blood volume and transmural pressure in a given segment of the vasculature. Given that around 70% of the total blood volume is contained in the venous vasculature, with compliance considerably higher than the arterial circulation, vascular capacitance in mammals is virtually a matter of the venous system (Pang, 2001; Rothe, 1993). The fact that the compliance ratio between arterial and venous vascular beds in the trout has been estimated to be at least 1:21 (Conklin and Olson, 1994) has lead previous investigators to assume that this assumption is justified for fish as well (Conklin et al., 1997; Olson et al., 1997; Zhang et al., 1995; Zhang et al., 1998). We therefore conclude that the increase in MCFP observed following pre-branchial occlusion was the result of an active α-adrenergic venoconstriction, serving to mobilize hemodynamically inactive venous blood into the stressed vascular compartment that builds up transmural pressure in the vascular system. Since capacitance is determined by the relationship between vascular compliance and tone it can only be fully described in terms of pressure–volume capacitance curves, not by a single number (Olson et al., 1997; Pang, 2001; Rothe, 1993; Zhang et al., 1998). In the present study no manipulations of blood volume were performed, as this is virtually impossible to achieve without stimulating (baro-) reflexes in intact animals. Capacitance curves could therefore not be obtained and a conclusive deduction of whether the observed changes in MCFP are due to changes in compliance and/or tone cannot readily be made (Olson et al., 1997; Pang, 2001; Rothe, 1993; Zhang et al., 1998). In dogs, however, reflex changes in venous capacitance are mainly mediated by changes in tone and not compliance (Rothe, 1993). Whether this can be extrapolated to the situation in fish is yet to be verified.

No significant difference in MCFP after post-branchial occlusion compared to the control was found in untreated fish (Fig. 2B). This is somewhat surprising considering that an adrenergic tonus on the venous capacitance vasculature is present in trout at rest (Zhang et al., 1998). Assuming that baroreceptors are exclusively restricted to the branchial circulation, an increase

in branchial blood pressure should theoretically result in a decreased adrenergic tone on capacitance vessels, thus decreasing mean circulatory filling pressure. However, after prazosin treatment a significant decrease in MCFP after post-branchial occlusion was unmasked, possibly due to a redistribution of blood into the splanchnic circulation (see below for further discussion). Similarly, if blood redistribution to the splanchnic circulation also occurred in the untreated fish, the measured MCFP would have been expected to be lower after dorsal aortic occlusion as well. This discrepancy can possibly be explained by the following factors: (1) The MCFP manoeuvre in itself requires a short ventral aortic occlusion. The compensatory reflex responses that might occur during this short circulatory arrest are generally assumed to be negligible (Rothe, 1993; Zhang et al., 1995). However, the possibility that the control system became somewhat super-sensitive during the post-branchial occlusion, which consequently caused the MCFP manoeuvre to counteract the response to the long occlusion, cannot be ignored. (2) Furthermore, baroreceptors located downstream to the post-branchial occlusion might have obscured the response. If so, low dorsal aortic blood pressure distal to the occlusion might have buffered the effect of an increased branchial blood pressure and/or splanchnic blood volume redistribution, thus leaving MCFP unaltered. The significant increase in control MCFP after prazosin treatment has been observed before in trout (E.S., unpublished) and in sea bass (E.S., A. P. Farrell, J. Altimiras, M.A. and G. Claireaux, submitted). This might seem strange considering that capacitance vessels in the trout are subjected to an α-adrenergic tonus at rest (Zhang et al., 1998). In other words, the capacitance curve was rotated counter-clockwise and shifted leftward due to a decreased tone and an increased compliance after prazosin treatment. The increase in MCFP after prazosin in the present study can be due to a number of factors such as passive fluid uptake when arterial pressure drops, and/or an increased compensation of some unknown vasoactive systems. In the study by Zhang et al. (1998), 20–40 min was allowed to elapse after prazosin treatment before the vascular capacitance curves were constructed. We suspect that this recovery might be too short to obtain a steady state condition. It is possible that the capacitance curve would be shifted leftward and gradually rotated clockwise as a consequence of passive fluid uptake after α-adrenergic blockade. This clearly remains to be substantiated. In the present study at least 1.5 h was applied to obtain α-adrenergic blockade, and indeed an initial decrease in P_{ven} was generally seen when prazosin was injected, but when the experiments were performed 1.5–2 h later central venous pressure had usually recovered to initial resting values or above.

Arterial resistance was probably also affected by a decrease in branchial blood pressure during pre-branchial occlusion (Fig. 3). In untreated animals dorsal aortic blood pressure was always initially increased after pre-branchial occlusion, compared to the value before occlusion. After prazosin treatment this response was completely absent, showing that an α-adrenergic response was responsible for this response. These findings are in line with previous findings in the anaesthetized carp (Ristori, 1970), but different from the study on the sea raven (Farrell, 1986), where no change in arterial conductance was seen after vascular compression.

Integration of chronotropic and vascular responses

The time course of the chronotropic and the vascular responses to manipulations of branchial blood pressure appear to differ considerably (Fig. 4). After pre-branchial occlusion the changes in heart rate start within seconds. By contrast, venous pressure first appears to plateau at MCFP before slowly increasing as a consequence of reflex venoconstriction (Rothe, 1993; Zhang et al., 1995, 1998). The physiological relevance of this would be that the changes in heart rate, and possibly also systemic resistance, mainly provide beat-to-beat fine-tuning of arterial blood pressure. This might in part serve as an effective means of protecting the delicate respiratory epithelium in the gills (Van Vliet and West, 1994). The process of blood mobilization from the venous circulation operates on a slightly larger time scale. In terrestrial animals subjected to gravitational forces, the necessity of venoconstriction during orthostatic challenges as a means of providing sufficient venous return is obvious. However, in aquatic animals that live in a near gravity-free environment, it is somewhat more difficult to visualize how such reflexes have evolved. Various explanations for the necessity of active venoconstriction in aquatic vertebrates have been presented. Ogilvy and DuBois (1982) noticed that bluefish *Pomatomus saltatrix* tolerated head-up tilting and could maintain arterial blood pressure. This ability was described as an adaptation of the vascular system to counteract hydrodynamic forces acting on the vasculature during swimming. It is also known that trout can actively mobilize venous blood in order to compensate for blood loss during haemorrhage (Duff and Olson, 1989). In mammals it is well known that MCFP increases when cardiac output is increased. This is believed to reflect an increase in the upstream venous (venular) driving pressure for venous return, which evidently has to increase when cardiac output is increased (Pang, 2001; Rothe, 1993). Cardiac output was the manipulated variable in the present study and therefore we cannot say whether it was increased or not. However, when cardiac output (and venous return) is increased during exercise in the European sea bass *Dicentrarchus labrax* there is a significant increase in MCFP, suggesting that regulation of venous capacitance is a general and highly important mechanism in the control of cardiac output in fish (E.S., A. P. Farrell, J. Altimiras, M.A. and G. Claireaux, submitted).

Location of baroreceptors

Although the barosensitive properties of the branchial circulation in fish are generally accepted, it is fascinating to note that the vascular and the cardiac responses to alterations in branchial blood pressure not necessarily operate in concert. For example, the lack of response (or decrease) in vascular capacitance with an associated bradycardia after post-branchial occlusion (Fig. 2A,B) in the untreated fish, raise the question of whether extra-branchial baroreceptors are involved in the control of cardiovascular homeostasis. It is tempting, but somewhat

premature, to speculate that gill-receptors mainly control the cardiac chronotropic limb of the baroreflex, as has previously been shown using open-loop techniques in anaesthetized fish (Mott, 1951; Ristori, 1970; Ristori and Dessaux, 1970), whereas additional baroreceptors located downstream possibly also mediate the vascular responses. It should be emphasized, however, that the consistent chronotropic responses to pre- and post-branchial occlusion in the present study, do not necessarily leave the gills as the sole location for baroreceptors controlling heart rate in fish. Potential post-branchial receptors would also have been affected by the occlusions.

Further studies are clearly needed. Due to anatomical constraints, open-loop studies similar to those used in toads, where gradual occlusion distal or proximal to various barosensitive areas enabled measurement of normalized gain, are possibly not applicable in the trout (for two comprehensive reviews, see Van Vliet and West, 1994; West and Van Vliet, 1994). Finding alternative experimental fish species where the large systemic vessels are surgically accessible could be the key to further understanding.

Methodological evaluation

Embolectomy catheters, which are normally used in clinical situations for treatment of arteriosclerosis, might be a valuable experimental tool in a variety of research situations in the field of comparative cardiovascular physiology. Preliminary studies revealed that an accurate placement of the occlusion balloon down the length of the dorsal aorta was crucial for a successful occlusion manoeuvre. If the balloon was positioned at the bifurcation of the celiacomesenteric artery, balloon inflation inevitably ruptured the vessel wall. Hence, in the experimental series the balloon was advanced further distal to the bifurcation where inflation did not induce any visible damage. This might have resulted in a net redistribution of blood into the splanchnic circulation during dorsal aortic occlusion. At least in mammals, the gut vasculature has a high capacitance, and the hepatic circulation a comparably large resistance (Rothe, 1993). Also in fish the entire gastrointestinal blood flow passes through the hepatic circulation before it empties into the central venous system (Thorarensen et al., 1991). A net shift of blood into the splanchnic circulation during dorsal aortic occlusion could therefore result in a decrease in mean circulatory filling pressure as measured in the central veins. In order to verify that branchial blood pressure actually increases during dorsal aortic occlusion, a ventral aortic catheter could have been used. Since the present experimental approach already demands fairly extensive instrumentation this was not done in an attempt to minimize surgical stress. Considering that 60–80% of cardiac output is distributed to the systemic arterial circulation at rest (Farrell et al., 2001), we feel confident that dorsal aortic occlusion produces enough increase in branchial blood pressure to stimulate potential barosensitive areas. A potential problem that arises when branchial outflow is transiently occluded is the fact that not only is branchial blood pressure increased, but presumably also blood pressure in the central nervous system. Although neither abnormal behavioural responses nor increased mortality, indicative of brain damage, were observed even after repeated occlusions, the potential effects of this cannot be ignored.

Concluding remarks

Bagshaw (1985) postulated that there has been an evolutionary transition amongst the vertebrates regarding the baroreflex. Regulation of cardiovascular homeostasis in the teleosts was claimed to be mainly heart-rate-based, whereas a more refined system including both cardiac and vascular properties first appears in the higher vertebrates. However, these arguments are not easy to reconcile, considering that our study clearly demonstrates that stimulation or unloading of baroreceptors in the trout not only results in chronotropic responses but also in profound changes in vascular capacitance and systemic resistance, very similar to the situation in mammals. As Jones and Milsom (1982) pointed out the baroreflex is likely to be a phylogenetically ancient cardiovascular trait that evolved well before vertebrates became subjected to gravitational forces when terrestrial habitats were colonized. Our findings further support this view by showing that both the cardiac and the vascular limbs of the baroreflex are important for the maintenance of cardiovascular homeostasis in fish.

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